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(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

(72) Inventors: FOSTEL, Jennifer, M.; 7413 10th Avenue, Kenosha, WI 53143 (US). GILES, Kellie, M.; 9526 Fairway Circle, Franklin, WI 53132 (US). TAYLOR, Alison; 2032 Flower Circle, Arlington Heights, IL 60004 (US). MCGONIGAL, Thomas, P.; 3211 Rugby Court, Waukegan, IL 60087 (US). SARTHY, Aparna, V.; 4836 Dorothy Court, Waukegan, IL 60089 (US).

(74) Agents: DANCKERS, Andreas, M. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

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(57) Abstract

The present invention provides an isolated and purified polynucleotide that encodes *Candida albicans* type I topoisomerase. Methods of making recombinant *C. albicans* topoisomerase I using those polynucleotides and host cells transformed with those polynucleotides are also provided. The present invention also provides a method for identifying compounds which inhibit the growth of fungal cells using the polynucleotide.

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CANDIDA TOPOISOMERASE I GENE

This application is a Continuation-in-Part Application of and claims the benefit of U.S. Provisional Application 60/005,989, filed October 27, 1995 and also claims the benefit of U.S. Application, filed October 25, 1996, entitled Candida Topoisomerase I Gene to inventors J.M. Fostel. A. Taylor, K.M. Giles, T.P. McGonigal and A. V. Sarthy.

Background of the Invention

Candida albicans (*C. albicans*) is an opportunistic pathogen which causes systemic candidiasis in immunocompromised patients. The patient pool at risk includes cancer patients undergoing chemotherapy, organ transplant recipients, patients infected with HIV, and other patients without a healthy immune response. This population is increasing in number annually, making infections by *Candida* spp. and other fungi an increasingly important clinical challenge (recently reviewed by Kerridge, 1995). It has been estimated that the majority of mycotic infections occurring in cancer patients are caused by *Candida albicans* and other species of *Candida* (Kerridge, 1995; Abi-Said and Anaissie, 1995), and that the excess mortality that can be attributed to candidemia is 38% (Wey *et al.*, 1988).

Current therapeutic agents available to combat *Candida* infections include amphotericin B, a polyene, and the family of azole compounds. Both classes of compounds inhibit fungal growth either through a direct or indirect interaction with an integral membrane component, ergosterol. Despite their effectiveness, however, neither compound completely satisfies therapeutic requirements. Amphotericin B, for example, is associated with adverse reactions in the host (Kerridge, 1995; Rex, *et al.*, 1994). The triazoles, while safer, may have reduced efficacy against a growing population of resistant *Candida* species. (Kerridge, 1995; DeMuri and Hostetter, 1995; Law *et al.*, 1994). Thus there is an unmet need for safe antifungal agents which also can be used against azole-resistant isolates of *Candida* spp.

It has been proposed that agents which adversely effect topoisomerases and in particular, type I topoisomerases, may be effective as antifungal agents (Figitt, 1989; Fostel *et al.*, 1992; Shen *et al.*, 1992). DNA topoisomerases are enzymes which modulate the topological structure of DNA and are classified into two classes based primarily on their mode of cleaving DNA. Type I DNA topoisomerases introduce a transient break in one strand of DNA, pass another strand through the nick and change the linking number (i.e. the number of times the DNA backbone strands intertwine) by one unit. Type II topoisomerases introduce concerted breaks in both DNA strands, pass another double-stranded DNA segment through the gap and change the linking number by two. This difference in

mechanism allows type II topoisomerases to catenate and decatenate covalently closed double-stranded DNA rings (Hsieh and Brutlag, 1980; Kreuzer and Cozzarelli, 1980), and also to knot and unknot covalently closed double-stranded circles (Liu *et al.* 1980; Hsieh, 1983). The type I topoisomerases, on the other hand, cannot catenate or decatenate double-stranded DNA unless a nick or gap is present in one of the circles (Tse and Wang 1980; Brown and Cozzarelli 1981).

In eukaryotic cells, topoisomerases participate in cellular processes which are associated with the separation of complementary DNA strands (i.e. replication, transcription, recombination and repair). Topoisomerases are also required for the segregation of daughter chromosomes during cytokinesis. Despite the important function these enzymes perform, type I topoisomerases are considered to be non-essential enzymes in that disruption or inhibition of their catalytic activity does not lead to cell death. (It has been proposed that functional topoisomerase II activity may replace or substitute for lost topoisomerase I activity). Thus, selective inhibition of the topoisomerase I catalytic activity of a particular fungal pathogen would not necessarily cure a host of that pathogen. Although type II topoisomerases could prove to be effective antifungal targets, to date, no compounds have been identified which selectively target the cleavage complex formed by the topoisomerase II enzymes of fungal origin.

It has been found however that type I topoisomerases can be adversely affected via a non-catalytic pathway, presumably by "stabilizing" a "cleavage complex" formed between a type I topoisomerase and DNA. In a normally replicating cell, a cleavage complex is characterized by a transient association between the topoisomerase I and DNA during which time the enzyme performs various catalytic functions (such as binding to and nicking a particular region of the DNA, passing a DNA segment through or around the break to change the linking number and ultimately resealing (i.e. repairing) the nick). Stabilizing agents are those chemical compounds which promote cell death through some manner of interaction with a topoisomerase I/DNA cleavage complex, although the nature of this interaction and the mechanism by which the agent induces cell death is unknown. Nitiss *et al.*, for example, have demonstrated the fungicidal effect of camptothecin (which stabilizes the topoisomerase I cleavage complex of *Saccharomyces cerevisiae* (Nitiss and Wang, 1988) and etoposide and amsacrine (which stabilize the topoisomerase II cleavage complex of *Saccharomyces cerevisiae* (Nitiss, 1992). Eng *et al.* have also demonstrated this effect in strains of *S. cerevisiae* and *Schizosacheromyces pombe* (Eng, *et al.*, 1988). Thus, it is reasonable to expect a novel class of stabilizing agents to have a similar effect on topoisomerase/DNA cleavage complexes of pathogenic fungi.

In order to minimize unwelcome effects on host tissue, it is important to determine if the fungal topoisomerases have sufficient biochemical differences from the human enzymes

to allow differential targeting by chemical agents. Fostel *et al.* (1992) isolated a type I topoisomerase from *Candida albicans* and characterized its response to known topoisomerase agents. The enzyme, *Candida* topo I, exhibited a differential response (relative to human topo I) to camptothecin and the aminocatechol A-3253 in being ten times less sensitive to camptothecin than human topo I and ten times more sensitive to A-3253 (Fostel *et al.*, 1992; Fostel and Montgomery, 1995). Others have also demonstrated a differential response by type I and type II topoisomerases to different agents (Figgitt *et al.*, 1989; Dykstra *et al.*, 1994).

Efforts to characterize biochemical differences between fungal topoisomerases and host topoisomerases have, however been hampered by the lack of easy and cost- effective methods to assay for such differences. For example, one common assay technique for measuring the effects of stabilizing agents requires the use of isolated and purified topoisomerase. Because the assay must be performed using stoichiometric amounts of enzyme, the tester must purify large quantities of enzyme in order to perform a minimal number of tests. Accordingly, there is a need to overproduce the enzyme in a time-conserving and cost-effective manner.

Furthermore, large quantities of enzyme or enzyme fragments may be desired to generate polyclonal or monoclonal antibodies. Such antibodies may then be used in affinity chromatography to facilitate purification of the enzyme. Large quantities of enzyme or enzyme fragments may also be desired to perform X-ray crystallography and other analytical studies.

The present invention solves these problems by identifying, isolating and expressing a type I topoisomerase gene from *C. albicans* in an expression system suitable for large-scale production. In addition, identification and isolation of a *C. albicans* type I topoisomerase gene allows for its expression in a variety of systems which facilitate purification of a *C. albicans* topoisomerase I encoded therefrom. A *C. albicans* type I topoisomerase gene may also used in a assay system for identifying compounds which inhibit the growth of fungi.

Summary of the Invention

In one aspect, the present invention provides an isolated and purified single- or double-stranded polynucleotide, typically DNA, having a nucleotide sequence comprising a nucleotide sequence selected from the group consisting of (a) the sense sequence of SEQ ID NO:1 from about nucleotide position 474 to about nucleotide position 2807; (b) a sequence complementary to the sequence of (a); (c) sequences that, when expressed, encode a polypeptide encoded by a sequence of (a); and d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b). A polynucleotide of the present invention

may also comprise the sense sequence of SEQ ID NO:1 from about nucleotide position 345 to about nucleotide position 2807 as well as the longer counterparts of (b), (c) and (d) above. A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

In another aspect, the present invention provides specific isolated and purified single- or double-stranded fragments of the above mentioned polynucleotide including the fragment counterparts of (b), (c) and (d) above. The specific fragments are from nucleotide position 474 to nucleotide position 923, nucleotide position 2199 to nucleotide position 2624 and nucleotide position 2655 to nucleotide position 2807 as numbered in FIG. 6.

In another embodiment, a DNA molecule of the present invention is contained in an expression vector. The expression vector preferably further comprises an enhancer-promoter operatively linked to the polynucleotide. In an especially preferred embodiment, the DNA molecule in the vector is one of the preferred sequences or fragments mentioned above.

Preferably, a topoisomerase I of the present invention is a recombinant topoisomerase I having the amino acid residue sequence of SEQ ID NO:2 or its subset SEQ ID NO:3. A preferred *C. albicans* topoisomerase I has about 821 or fewer amino acid residues and comprises the amino acid residue sequence of SEQ ID NO:3. Also useful are the shorter polypeptides encoded by the DNA fragments mentioned above.

In another aspect, the present invention provides a process of making *C. albicans* topoisomerase I comprising transforming a host cell with an expression vector that comprises a polynucleotide of the present invention, maintaining the transformed cell for a period of time sufficient for expression of the topoisomerase I, and recovering the topoisomerase I. Preferably, the host cell is an eukaryotic host cell such as a mammalian, yeast or fungal cell, or a bacterial cell. Especially preferred host cells are *Candida spp.*, *S. cerevisiae* and *E. coli*. The present invention also provides a topoisomerase I made by a process of this invention. A preferred such topoisomerase I is recombinant *C. albicans* topoisomerase I.

In another aspect, the present invention provides for a host cell transformed with a polynucleotide or expression vector of this invention. Preferably, the host cell is a yeast or fungal cell such as *S. cerevisiae* or *Candida spp.* or a bacterial cell such as *E. coli*.

The present invention further provides a method for identifying compounds having the ability to kill or inhibit the growth of fungal strains.

Brief Description of the Drawings

FIG. 1 shows the amino acid sequence used to design sets of degenerate oligonucleotide primers for isolating a 127 base pair (bp) *C. albicans* DNA fragment with

homology to eukaryotic topoisomerase I genes. In FIG. 1 and in all Figures hereinafter, the abbreviations "Hu, *Sc*, *Sp*, and *Dm*" have the following meanings: Hu = human, *Sc*= *Saccharomyces cerevisiae*, *Sp* = *Schizosaccharomyces pombe*, and *Dm* = *Drosophila melanogaster*. In FIG. 1, the Hu sequence represents amino acids 589-634 (D'Arpa *et al.*, 1988), the *Sc* sequence represents amino acids 516-560 (Thrash, *et al.*, 1985), the *Sp* sequence represents amino acids 561-605 (Uemura, *et al.*, 1987) and the *Dm* sequence represents amino acids 811-856 (Hsieh.*et al.*, 1992) of each species' respective topoisomerase I.

FIG. 2 shows a comparison of the sequence of the 127 bp *C. albicans* DNA fragment to nucleotides 2043-2180 of the *Saccharomyces cerevisiae* topoisomerase I gene.

FIG. 3 shows the predicted alignment of the polypeptide encoded by the 127 bp *C. albicans* DNA fragment with the amino acid sequences of genes encoding type I topoisomerases from other eukaryotic species. In FIG. 3 and in all Figures hereinafter, the abbreviations "*Ca*, *Um*, *Mm*, *Xl*, and *At*" have the following meanings: *Ca* = *Candida albicans*, *Um* = *Ustilago maydis*, *Mm* = *Mus musculus*, *Xl* = *Xenopus laevis*, and *At* = *Arabadopsis thaliana*. In FIG. 3, the *Ca* sequence represents the amino acids encoded by the insert in plasmid pKG1, *Sc* represents amino acids 514-561 (Thrash *et al.*, 1985), *Sp* represents amino acids 559-606 (Uemura *et al.*, 1987), Hu represents amino acids 587-635 (D'Arpa *et al.*, 1988), *Um* represents amino acids 557-616 (Gerhold *et al.*, 1994), *Mm* represents amino acids 589-637 (Baumgartner *et al.*, 1994), *Xl* represents amino acids 643-691 (Pandit and Sternglanz, 1992), *Dm* represents amino acids 809-858 (Hsieh *et al.*, 1992), and *At* represents amino acids 733-780 (Kieber and Signer, 1990) of each species respective topoisomerase I.

FIG. 4 shows the amino acid sequence used to design a set of degenerate oligonucleotide primers for isolating a 1044 bp *C. albicans* DNA fragment with homology to eukaryotic topoisomerase I genes. In FIG. 4, Hu represents amino acids 273-282, *Mm* represents amino acids 275-284, *Dm* represents amino acids 496-505 and *Sc* represents amino acids 201-210.

FIG. 5 shows the sequence of the cloned 1044 bp *C. albicans* DNA fragment.

FIG. 6(a-j) shows the complete double-stranded sequence of *C. albicans* topoisomerase I gene including upstream and downstream gene sequences and the predicted polypeptide encoded therefrom.

FIG. 7(a-b) shows the comparative homology in amino acids between C. *albicans* topoisomerase I and human topoisomerase I (d'Arpa, *et al.*, 1988).

FIG. 8 shows the nucleotide sequence of an adapter DNA which carries the 5' and 3' ends of the *C. albicans* topoisomerase I gene.

FIG. 9 shows a schematic diagram of the construction of plasmid pVT100-UCT7-7.

Detailed Description of the Invention

I. The Invention

The present invention provides isolated and purified polynucleotides that encode a type I topoisomerase from *Candida albicans*, fragments thereof, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making *C. albicans* topoisomerase I using those polynucleotides and vectors, and isolated and purified recombinant *C. albicans* topoisomerase I and polypeptide fragments thereof. The present invention also provides a method for identifying compounds having antifungal activity using polynucleotides that encode a type I topoisomerase from *C. albicans*.

II. TOP1 Polynucleotides

In one aspect, the present invention provides an isolated and purified polynucleotide that encodes a type I topoisomerase from the pathogenic fungus, *Candida albicans* (*C. albicans*). Hereinafter, the abbreviation "TOP1" is used to designate a gene or polynucleotide sequence encoding a type I topoisomerase; the abbreviation "topo I" is used to designate a polypeptide or an amino acid sequence encoded by a TOP1. A polynucleotide of the present invention that encodes *C. albicans* topo I is an isolated and purified polynucleotide having a nucleotide sequence which comprises a nucleotide sequence selected from the group consisting of

(a) the sense sequence of SEQ ID NO:1 from about nucleotide position 345 or 474 to about nucleotide position 2807,

(b) a sequence complementary to the sequence of (a),

(c) sequences that, when expressed, encode a polypeptide encoded by the sequence of (a), and

(d) analogous sequences that hybridize under stringent conditions to the sequences of (a) or (b). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

The nucleotide sequence and deduced amino acid residue sequence of *Candida albicans* topo I are set forth in SEQ ID NO:1 and SEQ ID NO:2 shown in FIG. 6. The nucleotide sequence of SEQ ID NO:1 is a full-length DNA clone of the gene encoding *C. albicans* topo I and is intended to represent both the sense strand (shown on top) and its complement (i.e. the strand shown below the top strand in FIG. 6). In eukaryotic cells, translation of RNA initiates at an AUG codon coding for methionine (which, in the *C. albicans* topo I shown in FIG. 6, corresponds to nucleic acid position 474). However, the

open reading frame (ORF) of *C. albicans* TOP1 begins at about nucleotide position 345. Thus SEQ ID NO:1 contains two "coding regions" (hereinafter CR1 and CR2) which, in FIG. 6, correspond to nucleotide positions 345-2807 and 474-2807, respectively, either of which may encode the native topo I enzyme. SEQ ID NO:2 is the deduced amino acid residue sequence encoded from CR1 and represents a polypeptide which is 821 amino acids in length. In FIG. 6, SEQ ID NO:2 starts with the amino glutamic acid (E) at position -43 and ends with the amino acid phenylalanine (F) at position 821. SEQ ID NO:3 is the deduced amino acid sequence encoded from CR2 and represents a polypeptide which is 778 amino acids in length. In FIG. 6, SEQ ID NO:3 begins with methionine (M) at position +1 and ends with pheylalanine (F) at position 821.

The present invention also contemplates shorter polynucleotide sequences or fragments of CR1 and CR2 which may or may not have topo I catalytic activity. More specifically, a polynucleotide of the present invention is also an isolated and purified single- or double-stranded polynucleotide having a nucleotide sequence which comprises a nucleotide sequence selected from the group consisting of (a) the sense sequence of SEQ ID NO:1 from about nucleotide position 474 to about nucleotide position to about nucleotide position 923, the sense sequence of SEQ ID NO:1 from about nucleotide position 2199 to about nucleotide position 2624 and the sense sequence of SEQ ID NO:1 from about nucleotide position 2655 to about nucleotide position 2807. The invention is also intended to encompass the fragment counterparts of (b), (c) and (d) mentioned above.

The present invention also contemplates analogous DNA sequences which hybridize under stringent hybridization conditions to the DNA sequences set forth above. Stringent hybridization conditions are well-known in the art and define a degree of sequence identity greater than about 80%-90%. The modifier "analogous" refers to those nucleotide sequences that encode polypeptides having only conservative differences and which retain the conventional characteristics and activities of a topoisomerase I; eg. nicking DNA, changing the DNA's linking number and resealing the nick. The present invention also contemplates naturally-occurring allelic variations and mutations of the DNA sequences set forth above so long as those variations and mutations code, on expression, for a topo I of this invention as set forth hereinafter.

As is well-known in the art, because of the degeneracy of the genetic code, there are numerous other DNA and RNA molecules that can code for the same polypeptides as those encoded by CR1, CR2 and fragments thereof. The present invention, therefore, contemplates those other DNA and RNA molecules which, on expression, encode for the polypeptide of SEQ ID NO:2, its subset SEQ ID NO:3 or fragments thereof. Having identified the amino acid residue sequence encoded by *C. albicans* TOP1, and with knowledge of all triplet codons for each particular amino acid residue, it is possible to

describe all such encoding RNA and DNA sequences. DNA and RNA molecules other than those specifically disclosed herein, which molecules are characterized simply by a change in a codon for a particular amino acid, are within the scope of this invention.

As is well-known in the art, codons constitute triplet sequences of nucleotides in mRNA molecules and as such, are characterized by the base uracil (U) in place of base thymidine (T) (which is present in DNA molecules). A simple change in a codon for the same amino acid residue within a polynucleotide will not change the structure of the encoded polypeptide. By way of example, it can be seen from SEQ ID NO:1 (see FIG. 6) that a TCA codon for serine (i.e. UCA in mRNA) exists at nucleotide positions 318-320 and again at positions 408-410 and 411-413. However, it can also be seen from that same sequence that serine can be encoded by an AGT codon (see eg. nucleotide positions 438-440) and a TCG codon (see e.g., nucleotide positions 462-464). Substitution of the latter codons for serine with the TCA codon for serine, or visa-versa, does not substantially alter the DNA sequence of SEQ ID NO:1 and results in expression of the same polypeptide. In a similar manner, substitutions of the recited codons with other equivalent codons can be made in a like manner without departing from the scope of the present invention.

Furthermore, it should be noted that, in *C. albicans*, the CUG codon has been shown to encode serine rather than leucine (see Santos and Tiute, 1995), although this phenomenon is not seen in all *Candida* species or in other fungi (such as *S. cerevisiae*). Thus, a recombinant *C. albicans* TOP1 of the present invention expressed in a *C. albicans* strain would most likely encode a topo I having a serine residue at amino acid position 407 (Xaa) in SEQ ID NO:2 and 321 in SEQ ID NO:3 (corresponding to the CUG at nucleotide position 1434 and amino acid "X" at position 321 in SEQ ID NO:1). In contrast, expression of *C. albicans* TOP1 in another *Candida* or other fungal species could result in placement of a leucine residue at this same position. Thus, the present invention contemplates a topo I having either a serine or leucine residue at the Xaa position depending on the expression system used.

A polynucleotide of the present invention can also be an RNA molecule. A RNA molecule contemplated by the present invention is complementary to or hybridizes under stringent conditions to any of the DNA sequences set forth above. Exemplary and preferred RNA molecules are mRNA molecules that encode a topo I of this invention.

The present invention also contemplates oligonucleotides from about 15 to about 50 nucleotides in length, which oligonucleotides serve as primers and hybridization probes for the screening of DNA libraries and the identification of DNA or RNA molecules that encode *C. albicans* topo I. Such primers and probes are characterized in that they will hybridize to polynucleotide sequences encoding topo I. An oligonucleotide probe or primer contains a nucleotide sequence of at least 15 nucleotides that is identical to or complementary to a

contiguous sequence of a topoisomerase I polynucleotide of the present invention. Thus, where an oligonucleotide probe is 25 nucleotides in length, at least 15 of those nucleotides are identical or complementary to a sequence of contiguous nucleotides of a TOP1 of the present invention. Exemplary TOP1 polynucleotides of the present invention are set forth above.

An oligonucleotide primer or probe of the present invention can be prepared using standard procedures well-known in the art. A preferred method of polynucleotide synthesis is via cyanoethyl phosphoramidite chemistry. A detailed description of the preparation, isolation and purification of polynucleotides encoding *C albicans* topo I is set forth in the Examples below.

III. *C. albicans* topo I Polypeptides

In another aspect, the present invention provides a recombinant type I topoisomerase from *C albicans*. A *C. albicans* topo I of the present invention is a polypeptide of about 821 or fewer amino acid residues. As set forth in FIG. 7, forms of type I topoisomerases have been identified in various species with from 314 to 972 amino acid residues. The various forms of topo I are characterized by a moderate degree of sequence identity. By way of example, the identity between human and *C. albicans* enzyme is 45% at the amino acid level.

When the amino acid residue sequence encoded by *C. albicans* TOP1 was compared against other known amino acid residue sequences using a database searching algorithm, only moderate sequence similarity was found. The varying degree of identity among type I topoisomerases from various species allows for definition of the *C. albicans* topo I amino acid residue sequence by the regions lacking identity. Thus, in one embodiment, a *C. albicans* topo I is an isolated and purified polypeptide of about 821 or less amino acid residues, comprising at least one of the following amino acid residue sequences:

a) from residue position 1 to residue position 150 of SEQ ID NO:3;

b) from residue position 297 to residue position 529 of SEQ ID NO:3;

c) from residue position 573 to residue position 717 of SEQ ID NO:3; and

d) from residue position 728 to residue position 778 of SEQ ID NO:3.

Preferably, a topo I of the present invention comprises two or more of the above sequences. Most preferably, the topo I has all of the above sequences.

More preferably, a topo I of the present invention is a recombinant topo I from *C. albicans*. Although generally, eukaryotic polypeptides are known to begin with a methionine residue, the present invention also contemplates a recombinant topo I encoded by the complete ORF of *C. albicans* TOP1. Thus, recombinant *C. albicans* topo I can be defined as a polypeptide of about 821 or less amino acid residues comprising the amino acid

residue sequence of SEQ ID NO:2. A preferred recombinant *C. albicans* topo I has the amino acid residue sequence of SEQ ID NO:3.

The present invention also contemplates amino acid residue sequences that are substantially duplicative of the sequences set forth herein such that those sequences demonstrate like biological activity when compared to disclosed sequences. Such contemplated sequences include those sequences characterized by a minimal change in amino acid residue sequence or type (e.g., conservatively-substituted sequences) which insubstantial change does not alter the fundamental nature and biological activity of *C. albicans* topo I.

It is well known in the art that modifications and changes can be made in the structure of a polypeptide without substantially altering the biological function of that peptide. For example, certain amino acids can be substituted for other amino acids in a given polypeptide without any appreciable loss of function. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like.

As detailed in United States Patent No. 4,554,101, incorporated herein by reference, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4). It is expected that an amino acid residue can be substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0) and still result in a biologically equivalent polypeptide.

In a similar manner, substitutions can be made on the basis of similarity in hydropathic index. Each amino acid residue has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those hydropathic index values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5). In making a substitution based on the hydropathic index, a value of within plus or minus 2.0 is preferred.

A topo I of the present invention has numerous uses. By way of example, such a polypeptide can be used in assays which identify compounds that stabilize the cleavage complex of topo I and DNA. A topo I polypeptide can also be used to design compounds that interact with the cleavage complex of topo I and DNA. More specifically, the topo I or a portion of the topo I can be crystallized and used for designing drugs that stabilize the cleavage complex formed between the topo I and DNA.

In addition, a topo I of the present invention or fragments thereof may used to

produce antibodies that immunoreact specifically with *C. albicans* topo I. Such antibodies may then be used to isolate and purify *C. albicans* topo I. Means for producing antibodies are well known in the art. An antibody directed against *C. albicans* topo I can be a polyclonal or a monoclonal antibody.

Antibodies against *C. albicans* topo I can be prepared by immunizing an animal with a topo I polypeptide of the present invention. Means for immunizing animals for the production of antibodies are well-known in the art. By way of an example, a mammal can be injected with an inoculum that includes a polypeptide as described herein above. The polypeptide can be included in an inoculum alone or conjugated to a carrier protein such as BSA or keyhole limpet hemocyanin (KLH). The polypeptide can be suspended, as is well-known in the art, in an adjuvant to enhance the immunogenicity of the polypeptide. Sera containing immunologically active antibodies are then produced from the blood or other fluid (eg. ascites) of such immunized animals using standard procedures well-known in the art.

The identification of antibodies that immunoreact specifically with *C. albicans* topo I is made by exposing sera suspected of containing such antibodies to a polypeptide of the present invention to form a conjugate between antibodies and the polypeptide. The existence of the conjugate is then determined using standard procedures well-known in the art.

A topo I polypeptide of the present invention or fragments thereof may also be used to prepare monoclonal antibodies against topo I and used as a screening assay to identify such monoclonal antibodies. Monoclonal antibodies are produced from hybridomas prepared in accordance with standard techniques such as that described by Kohler *et al.*. Briefly, a suitable mammal (e.g., BALB/c mouse) is immunized by injection with a polypeptide of the present invention. After a predetermined period of time, splenocytes are removed from the mouse and suspended in a cell culture medium. The splenocytes are then fused with an immortal cell line to form a hybridoma. The formed hyridomas are grown in cell culture and screened for their ability to produce a monoclonal antibody against topo I. Screening of the cell culture medium is made with a polypeptide of the present invention.

IV. Method of Making *C. albicans* topo I.

In another aspect, the present invention provides a process of making *C. albicans* topo I. In accordance with that process, a suitable host cell is transformed with a polynucleotide of the present invention. The transformed cell is maintained for a period of time sufficient for expression of the topo I; the topo I is then recovered.

Means for transforming host cells in a manner such that those cells produce recombinant polypeptides are well-known in the art. Briefly, a polynucleotide that encodes

the desired polypeptide is placed into an expression vector suitable for a given host cell. That vector can be a viral vector, phage or plasmid and may be one which either replicates autonomously within the host cell or integrates into the host cell chromosome. In a preferred embodiment, a host cell used to produce topo I is an eukaryotic host cell and an expression vector is an eukaryotic expression vector (i.e., a vector capable of directing expression in a eukaryotic cell). Such eukaryotic expression vectors are well known in the art. An especially preferred eukaryotic host cell is an *S. cerevisiae* or *C. albicans*. A preferred expression vector is a vector capable of directing expression in *S. cerevisiae* or *C. albicans*.

In another preferred embodiment, the host cell is a bacterial cell. An especially preferred bacterial cell is an *E. coli*. Thus, a preferred expression vector is a vector capable of directing expression in *E. coli*.

A polynucleotide of an expression vector of the present invention is preferably operatively associated or linked with an enhancer-promoter. A promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription begins. That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region or a promoter of a generalized RNA polymerase transcription unit. Preferred promoters are those which are operative in bacteria such as a *lac* promoter. Even more preferred promoters are those which are operative in yeast or fungi. Especially preferred promoters are an ADH1 or GAL 10 promoter of *S. cerevisiae* or a *C. albicans* promoter.

Another type of transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from a transcription start site so long as the promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" or its grammatical equivalent means that a regulatory sequence element (e.g. an enhancer-promoter or transcription-terminating region) is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well-known in the art.

An enhancer-promoter used in an expression vector of the present invention can be any enhancer-promoter that drives expression in a host cell. By employing an enhancer-

promoter with well-known properties, the level of expression can be optimized. For example, selection of an enhancer-promoter that is active in specifically transformed cells permits tissue or cell specific expression of the desired product. Still further, selection of an enhancer-promoter that is regulated in response to a specific physiological signal can permit inducible expression.

A coding sequence of an expression vector is operatively linked to a transcription-terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA). Enhancer-promoters and transcription-terminating regions are well-known in the art. The selection of a particular enhancer-promoter or transcription-terminating region will depend, as is also well-known in the art, on the cell to be transformed.

The present invention also comtemplates the use of expression vectors which facilitate purification of a desired polypeptide. For example, a gene encoding the desired polypeptide may be cloned into an expression vector which, when expressed, produces the polypeptide fused or linked to a chemical or biological tag. A tag may be any chemical or biological compound or fragment thereof capable of binding to a specific substrate or receptor. Thus, tags serve to facilitate purfication of a fusion product (i.e. tag/polypeptide) via specific binding of the tag portion to its receptor or substrate. Preferably the tag is fused to the polypeptide in a manner that permits it to be cleaved from the polypeptide after purification. In illustration, *C. albicans* TOP1 could be cloned into a pGEX vector (Pharmacia Biotech, Inc., Piscataway, New Jersey) which, when placed in a suitable host, would express a fusion protein of *C. albicans* topo I and glutathione S-transferase (GST). The fusion protein could then be purified by affinity chromatography using glutathione sepharose 4B (which binds to the GST portion of the fusion product) and the topo I enzyme cleaved from the GST tag using a site-specific protease, the recognition sequence of which is located upstream from the topo I. Other commercially available tags could also be used and are well-known to those of ordinary skill in the art.

The present invention also contemplates a host cell transformed with a polynucleotide or expression vector of this invention. Means for transforming cells and polynucleotides and expression vectors used to transform host cells are set forth above. Preferably, the host cell is an eukaryotic host cell such as a yeast or fungal cell or a prokaryotic cell such as an *E. coli*.

V. Method of Identifying Stabilizing Agents of *C. albicans* topo I and DNA Cleavage

Complex

In another aspect, the present invention provides a method for identifying antifungal compounds and, more specifically, antifungal compounds which act as stabilizing agents of a *C. albicans* topo I and DNA cleavage complex. A "cleavage complex", as used herein, refers to a transient association between a topoisomerase and DNA during which time the enzyme performs various catalytic functions (such as binding to and nicking a particular region of the DNA, passing a DNA segment through or around the break to change the linking number, and ultimately resealing (i.e. repairing) the nick). The term "stabilizing agent" as used herein refers to a chemical compound which inhibits cell growth or promotes cell death through some manner of interaction with a topoisomerase I/DNA cleavage complex. The term "stabilize," or "stabilization" when used herein and in reference to a stabilizing agent and a cleavage complex, refers to that interaction of the stabilizing agent with the cleavage complex which leads to significant inhibition of cell growth and reproduction or, preferably, cell death. As shown by Nitiss and Wang (1988), the chemical compound camptothecin stabilizes the topo I/DNA cleavage complex of *Saccharomyces cerevisiae*, inducing fungal death. Notwithstanding this observation, the nature of the interaction between the stabilizing agent and the cleavage complex and the mechanism by which the agent induces cell death is unknown.

The present invention provides an assay method for identifying stabilizing agents of a *C. albicans* topo I/DNA cleavage complex. Assays for identifying stabilizing agents may be carried out both in whole-cell preparations and in *ex vivo* cell-free systems. In each instance, the assay target is a *C. albicans* topoisomerase I/DNA cleavage complex, wherein the *C. albicans* topoisomerase I is a *C. albicans* topo I of the present invention. It is expected that the assay methods of the present invention will be suitable for both small- and large-scale screening of test compounds, as well as in quantitative assays such as serial dilution studies wherein the target topo I/DNA cleavage complex is exposed to a range of test compound concentrations.

When the method of the present invention is carried out as a whole-cell assay, the target is an intracellular topo I/DNA cleavage complex and the entire, living fungal cell is exposed to the test compound under conditions normally suitable for growth. Such conditions, including essential nutrients, optimal temperatures and other parameters, depend upon the particular fungal strain being used and are well-known in the art. Stabilization of the topo I/DNA cleavage complex may be determined by observing the cell culture's growth or lack thereof; such observation may be made visually, by optical densitometric or other light absorption/scattering means, or by yet other suitable means, whether manual or automated.

In the above whole-cell assay, an observed lack of cell growth may be due to

stabilization of the target topo I/DNA cleavage complex or due to an entirely different effect of the test compound so that further evaluation is required to establish the mechanism of action and to determine whether the test compound is a specific stabilizing agent of the cleavage complex. Accordingly, and in a preferred embodiment of the present invention, the method may be performed as a paired-cell assay in a manner similar to that described by Housey in U.S. Patent No. 4,980,281, published December 25, 1990. In a paired-cell assay of the present invention, each test compound is separately tested against two different fungal strains, one having greater topo I activity than the other. Most preferably, such strains are isogenic to each other with respect to this particular trait, meaning that they are equivalent genetically except in their expression of TOP1. (By way of example, isogenic strains may differ with respect to the TOP1 gene by having a copy of a TOP1 gene not found in the other strain or by having multiple copies of a TOP1 gene wherein the other strain has only one copy). In this circumstance, those cells of a strain having greater topo I activity will be more susceptible to the lethal effects of a stabilizing agent than an isogenic strain of cells having lower topo I activity and thus will have comparatively greater mortality or inhibition of cell growth.

For preparing the above described paired-cell assay, one strain of cells may be altered by any manner of chemical or genetic modification to produce a modified strain having differential topoisomerase I activity relative to a second strain. For example, cells may be chemically modified by exposure to mutagenic agents (such as carcinogenic compounds or UV radiation) to produce a modified strain which underexpresses TOP1 relative to the unmodified parent. In this case, it is possible that such a strain and its parent would not be isogenic to each other since random chemical mutagenesis could affect other genes in addition to a TOP1 gene. However, a chemically modified strain could be further modified by genetic manipulation (to remove extraneous genetic changes by performing crosses to the parent strain and recovering progeny having reduced topo I) for use in a paired-cell assay; in such a case, the parent (i.e. without the additional chemical modification) would serve as an isogenic control. Alternatively, a TOP1 gene may be chemically modified *ex vivo* by exposure to a mutagenic agent and then reintroduced to a cell to produce a modified cell which expresses altered topo I activity relative to its unmodified parent. In yet another example, the activity of a topo I can be modified *in vivo* by the presence of a DNA binding agent (such as ethidium bromide) which can be introduced into the culture medium of one strain to alter the topo I activity of that strain relative to the same strain grown without the binding agent. Thus, the initial condition of the cells is not critical to the practice of a paired-cell assay; one need only be able to modify the cells in some manner that is not lethal and which generates a measurable difference in topo I activity.

A strain may also be genetically manipulated to alter the production of topo I in a particular cell. For example, a strain may be modified to either produce or over-produce topo I by cloning a *C. albicans* TOP1 of the present invention into a plasmid or suitable expression vector and transforming the cloned vector into a suitable host strain by means known to those of ordinary skill in the art. Suitable expression vectors and host cells are those previously described in Section IV above. Other preferred expression vectors are the non-integrative double-ARS shuttle vectors described by Pla *et al.* (1995) which are able to autonomously replicate in *C. albicans*.

For determining whether a compound acts as a fungicide or inhibitor of fungal growth, both the modified strain (harboring an expression vector comprising a TOP1 gene of the present invention) and the isogenic parental strain (harboring the expression vector only) would be exposed simultaneously to a compound of interest under essentially identical growth conditions. A compound which induces death of the modified strain or which inhibits that strain's growth would be a putative stabilizing agent.

Alternatively, a strain may be genetically modified to reduce the activity of a native topo I by any means known to disrupt a TOP1 coding sequence. For example, a cloned *C. albicans* TOP1 of the present invention can be used to disrupt or inactivate a native TOP1 within a host strain (i.e. *in vivo*). Techinques for performing gene disruption are well known to those of ordinary skill in the art, (see for example, Fonzi, W.A. and Irwin, M.Y., 1993 and Rothstein, R., 1991). As one example, targeted integration of a selectable marker into the chromosomal copy of a yeast or fungal TOP1 may be accomplished by first cloning the selectable marker gene into a single site within a coding sequence of *C. albicans* TOP1 (i.e. CR1 or CR2) or a fragment thereof. Selectable marker genes are those which confer upon a cell the ability to grow under a particular set of growth conditions (i.e. in selective medium). Representative examples of selective marker genes include but are not limited to genes which confer drug resistance, such as G418 and genes which confer upon a cell the ability to grow in the absence of an exogenously supplied essential nutrient.

When a fragment of a coding region is used, it should be of a such a length that the marker gene, after cloning, is flanked on each side by a sequence of the coding region that is at least about 250 base pairs in length. The cloned fragment is then transformed into the targeted host cell to induce recombination into the chromosomal copy, and then grown on selective media to identify recombinant cells. As a consequence, functional topo I will not be produced by that particular cell.

As another example, a non-functional topo I may be generated by genetically altering a chromosomal copy of a TOP1 through the process of gene replacement. Gene replacement may be accomplished by specifically altering the DNA sequence of a *C. albicans* TOP1 (so as to render the topo I encoded therefrom non-functional), subcloning the

altered sequence into a suitable vector capable of recombining in the yeast or fungal host, and introducing the vector containing the subcloned sequence into the appropriate host so that exchange of the wild-type allele with the mutated one will occur. The steps involved in gene replacement employ standard recombinant DNA techniques and are well known to those of ordinary skill in the art.

To identify stabilizing agents using modified cells having reduced or no topo I activity, both the modified strain and the parental strain would be exposed to a compound or compounds of interest under conditions suitable for growth. In this case however, the parental strain would be most susceptible to a stabilizing agent and would die or show inhibited growth as a result of exposure to the compound.

Any techniques for directly or indirectly measuring topo I protein or topo I activity are suitable for establishing differential topo I production between a modified strain and its parent. For example, quantitative analysis of topo I may be performed by preparing lysates of cells, partially purifying the topo I from the lysates and measuring topo I activity *ex vivo* (*cf.* Fostel *et al.*, 1992). Alternatively, when a modified strain is generated by the introduction of a multicopy plasmid containing a TOP1, the actual copy number of the plasmid in the modified strain may be quantified. As another example, a modified strain and its parent may be preliminarily tested for sensitivity to a known stabilizing agent such as camptothecin. Thus, the technique selected may vary depending upon the manner in which the modification is achieved but such techniques are either well known to those of ordinary skill in the art or are taught in the present specification.

The invention will be better understood in connection with the following examples, which are intended as an illustration of and not a limitation upon the scope of the invention. Both below and throughout the specification, it is intended that citations to the literature are expressly incorporated by reference.

EXAMPLE 1

Identification of *C. albicans* topo I Gene

1a. Isolation of a 127 bp DNA fragment from *C. albicans* having homology to eukaryotic type 1 topoisomerase genes

Genomic DNA was isolated from *C. albicans* strain ATCC 10321, by the method of Polaina and Adam (1991). Cells were grown in YEPD broth (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose, all from Difco, Detroit, MI) to a density of approximately 8 absorbance units, harvested, washed in TGE (20 mM KCl, 1 mM EDTA, 20 mM Tris pH

7.4, 10% glycerol), and frozen at -70°. Cells were thawed, resuspended in lysis buffer (3% sodium lauryl sarcosinate (SDS), 10 mM Tris-HCl pH 7.5, 1 mM EDTA), extracted with phenol/chloroform and precipitated with ethanol. The resulting DNA was used as a template for the polymerase chain reaction (PCR; Saiki *et al.*, 1988). Two sets of degenerate primers for PCR were selected based on conserved regions in other eukaryotic type I topoisomerases (Hsieh *et al.*, 1992) as shown in FIG. 1. New sites for restriction endonucleases Xba I and Bam H1 were also introduced into the primers to facilitate subcloning. The primer sets used were:

5'-CTCTAGATTYMGNACNTAYAAYGC-3' SEQ ID NO:4:

5'- TGGATCCYTGRTGRTTRCANARDAT-3' SEQ ID NO:5:

The symbols shown in these primers (and in all other primers mentioned herein) have the meanings shown in Table 2.

TABLE 2

Symbol	Base
A	Adenine
C	Cytosine
D	any base but cytosine (not C)
G	Guanine
M	A or C only
N	any base
R	A or G only
T	Thymine
U	Uracil
Y	C or T/U only

The PCR reaction was carried out using reagents from Perkin Elmer (Norwalk, CN), according to the manufacturer's recommendations, using 2 μg genomic *C. albicans* DNA and approximately 1 nmole of each of SEQ ID NO:4 and SEQ ID NO:5 (synthesized by Northwestern University, Evanston, IL). The reactions were incubated at 45°C for 1 minute, raised to 72°C over a two minute interval, held at 72°C for 1 minute, then at 94°C for 1 minute. This pattern was repeated for 50 cycles. The resulting DNA products were separated using a TBE-urea 17-26% polyacrylamide gel (Enprotech, Natick, MA); each band was characterized according to the following method:

The DNA band of interest was excised from the gel using the "crush and soak method" (Sambrook *et al.*, 1982). Briefly, the fragment of gel containing the DNA of interest was placed in a tube, crushed with a sterile pipette, and incubated at 37°C for 16 hours in elution buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1% SDS, 1 mM EDTA, pH 8.0). The excised DNA was incubated with the Klenow fragment of DNA

polymerase I (Boehringer Mannheim, Indianapolis, IN) in the presence of the four nucleotide triphosphates to fill in single-stranded termini, then extracted with phenol/chloroform and precipitated with ethanol. Plasmid pBSK+ (Stratagene GmbH, Heidelberg, Germany) was cut with EcoRV (GIBCO BRL, Gaithersburg, MD). This and other restriction digests were performed in the manufacturer's recommended reaction buffer, and unless otherwise indicated, all restriction and modifying enzymes were obtained from this same source. After digestion, the plasmid DNA was extracted with phenol/chloroform and precipitated with ethanol. The excised DNA was ligated to the plasmid using T4 DNA ligase in the manufacturer's reaction buffer, at 17° C for 16 hours. The resulting DNA was used to transform competent Maximum Efficiency DH5αF'IQ *E. coli* (GIBCO BRL, Gaithersburg, MD), following the manufacturer's recommended protocol, and an aliquot of 100 µL of the cells was plated on LB-agarose (Lennox broth; from Micro Diagnostics, Lombard, IL) containing 150 µg/mL ampicillin. Prior to use, 35 µL of a 50 mg/mL X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside; Sigma, St. Louis MO) solution in dimethyl formamide and 20 µL of a 100 mM IPTG (Isopropyl β-D-thiogalactopyranoside; Sigma, St. Louis, MO) solution in water were spread on the plate. DNAs from clones carrying inserts were identified on the basis of altered mobility on agarose gels.

The sequence of the inserts was determined using commercial primers from Stratagene. The primers,

5'-AATTAACCCTCACTAAAGGG-3' SEQ ID NO:6

5'-GTAATACGACTCACTATAGGGC-3' SEQ ID NO:7

are homologous to the T3 and T7 promoter sequences respectively, in the pBSK+ plasmid. Primers were end-labeled with $\gamma^{32}P$ using polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN) in the buffer provided by the manufacturer, then extended with Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN) using dideoxy-sequencing reagents and the method obtained from Promega (Madison, WI). The sequence of the insert from one clone designated 6AB was determined and is given in FIG. 2 (see "*Ca*"). This insert sequence was also compared to a yeast TOP1, i.e. to nucleotides 2034-2180 of the *Saccharomyces cerevisiae* TOP1 (see sequence "*Sc*" in FIG. 2) using the BESTFIT program (GCG, Wisconsin Software package, Madison WI) and the DNA Strider 1.2 program (C. Marck, Centre d'Etudes de Saclay, Gif-Sur-Yvette Cedex, France).

The pBSK+ plasmid with the 6AB fragment inserted was renamed pKG1. A BLAST search (GCG, Wisconsin Software package, Madison WI) of DNA sequences in the GENEMBL database identified other topoisomerase genes as those sequences with the highest score (i.e. having the greatest percent homology to the 6AB insert). An alignment of the predicted *C. albicans* protein sequence encoded by 6AB with other homologous regions of type I topoisomerases from other species is shown in FIG. 3.

Hybridization to *C. albicans* genomic DNA using the method of Southern (1975) revealed the presence of a single copy sequence homologous to pKG1.

1b. Isolation of a 1044 bp DNA fragment having homology to eukaryotic type I topoisomerase genes

A new set of degenerate primers, based on the sequence 5'-AARAAYTTYTTYAARGA-3' (SEQ ID NO:8) was constructed for the purpose of synthesizing an additional portion of the *C. albicans* topo I gene via PCR. The design of SEQ ID NO:8 was based on anticipated homology to other eukaryotic type I topoisomerases as shown in FIG. 4. SEQ ID NO:8 and a homologous primer, 5'-AGCCACCGTTCTATTGGCAGC-3' (SEQ ID NO:9) derived from the 6AB sequence, were used in a PCR reaction with genomic *C. albicans* DNA as template. The reaction was carried out using reagents from Perkin Elmer, according to the manufacturer's recommendations, with 2 μg genomic *C. albicans* DNA and approximately 1 nmole of each primer. The reactions were incubated at 45° C for 2 minutes, raised to 72° C over a one minute interval, held at 72° C for 3 minutes, then 94° for 1 minute. This pattern was repeated for 50 cycles. A DNA species having the anticipated size of approximately 1 kb was produced.

The PCR products were electrophoresed on an 0.8% low melt agarose gel (LMPA, BRL, Gaithersberg, MD), and the gel slice containing the DNA of interest was excised, melted and subcloned directly into pGEM-T ™ (Promega, Madison, WI) using the manufacturer's recommended conditions. The resulting plasmid was verified by restriction analysis, and termed pAT100.

The insert of *C. albicans* DNA in pAT100 was sequenced using a fmol DNA sequencing kit obtained from Promega (Madison, WI), with primers homologous to the T3 and M13 sequences in the vector, i.e. SEQ ID NO:6 and SEQ ID NO:10 (5'-GTAAAACGACGGCCAGT-3'). The insert sequence obtained (SEQ ID NO:11) is given in FIG. 5.

1c. Comparative homology of the 1044 bp DNA fragment to other eukaryotic type 1 topoisomerase genes

The 1044 bp sequence was used as a probe for a search of the GENEMBL (European Molecular Biology Organization) sequence database using the BLAST routine (GCG Wisconsin software package, University of Wisconsin, Madison, WI). BLAST uses the algorithm of Altschul *et al.* (J. Mol. Biol. 215: 403-410 (1990)) to search the database entries

for similarity to the probe sequence. The results showed that the highest scoring hits of the insert sequence were to other eukaryotic type I topoisomerases, suggesting that the 1044 bp fragment was indeed part of a *C. albicans* topoisomerase I gene.

EXAMPLE 2

Isolation of *C. albicans* topo I gene

2a. Construction of a *C. albicans* Genomic Library

C. albicans genomic DNA was prepared according to Olson *et al.* (1979). Cells were grown overnight in 500 mL of Mock YEPD liquid medium (purchased from BIO101, Vista, CA) from a 3 mL inoculum. The culture was divided into four equal volumes, harvested by centrifugation and washed with water two times. Approximately 6 g wet weight of cells were harvested in total. The 1.5 g aliquots of cells were then resuspended in 4 mL of SCE (1.0 M sorbitol, 0.1 M sodium citrate, 0.06 M EDTA, pH 7) to which was added 2 mg of zymolyase 5000 and 30 µL of ß-mercaptoethanol (Sigma, St. Louis, MO) to cause the formation of spheroplasts by degradation of the cell wall. Spheroplasting was followed microscopically by treating an aliquot of cells with 10% SDS. Spheroplasted cells were then added to 7 mL of lysis solution (3% SDS, 0.5 M Tris-HCL. pH 9.0, 0.2 M EDTA), incubated at 65° C for 15 minutes, and cooled rapidly to room temperature. The lysates were layered on top of 50/20/15% sucrose solutions which also contained 0.8 M NaCl, 20 mM Tris-HCL pH 8.0, and 10 mM EDTA. Gradients were centrifuged at 26,000 rpm for 2.5 hours at 20°C using a Beckman SW28 rotor. DNA was collected from the top of the 50% sucrose pad, extracted by rolling very gently with phenol/chloroform for 20 minutes, dialyzed against TE buffer (10mM Tris HCl pH 7.5, 5.0 mM EDTA) and then concentrated against solid sucrose.

Approximately 200 µg of this *C. albicans* genomic DNA was used by Stratagene (La Jolla, CA) to construct a genomic DNA library in λFIX®II (Stratagene, La Jolla, CA). The λFIX®II vector accepts inserts of size 9-20 kb, so assuming an average insert size of 15 kb, approximately 1000 clones would be expected to contain one genome equivalent of *C. albicans* DNA (genome size 15 megabases). This range of insert size was also considered to be the most useful size for isolation of a full length gene product from *C. albicans*, as the topoisomerase I gene would be expected to span at least 2.5 kb, assuming similarity to the *S. cerevisiae* TOP1 gene. The *C. albicans* genomic DNA was partially digested with Sau3A and size-selected by gel electrophoresis. It was then ligated into the λFIX®II vector following digestion with Xho I and treatment to a partial fill-in reaction, then grown in a P2

host (XL-1 Blue MRA(P2)). Both unamplified and 1x amplified libraries prepared according to this procedure were obtained from Stratagene.

2b. Identification of Phage with Homologous DNA Insert

An aliquot of the lx amplified *C. albicans* genomic library was used to infect XL1-Blue MRA (P2) according to the manufacturer's suggested conditions. Approximately 25,000 plaques were plated on NZCYM agar (GIBCO, BRL, Gaithersburg, MD) and incubated for 8 hours at 37 °C. Three plates were prepared, and the protocol given by Stratagene was followed to process the plaques for subsequent screening. Filters containing the plaques were prehybridized in PIPES buffer, 50% formamide, 100 μg salmon sperm DNA and 0.5% SDS. The filters were prehybridized at 42 °C for 2 hours followed by hybridization at 42 °C for 24 hours with a probe prepared as described below.

The probe was prepared for hybridization by first digesting pAT100 with Pst1 and Sph1. The digested DNA was electrophoresed on an 0.8% low melt agarose gel (LMPA, GIBCO BRL, Gaithersburg, MD), the 1 kb band was excised and labeled with γ^{32}P-dNTP using a Mega Prime labeling kit obtained from Amersham (Arlington Heights, IL). After hybridization, the filters were washed two times with 2X SSC for 15 minutes at 65 °C (1X SSC is 0.15M NaCl, 0.015M Na citrate) followed by washes for 30 minutes at 65°C with 2X SSC, 0.1% SDS and then 0.2X SSC, 0.1% SDS. Filters were air dried and exposed at -70 °C to Kodak X-AR ™ film (Eastman Kodak, New Haven, CT). Films were developed according to manufacturer's recommended conditions.

Thirty positive plaques from a total of approximately 45 were purified by one round of plaque purification using the labeled probe derived from pAT100. Two of the plaques yielding the strongest hybridization signal, #3 and #26, were arbitrarily selected for a third round of plaque purification following which DNA was prepared from clone #26 using a Wizard Lambda DNA Purification kit from Promega (Madison, WI).

EXAMPLE 3

Characterization of *C. albicans* topo I gene

3a. Sequencing Methodology

New sequencing primers were prepared to the 5' and 3' ends of the pAT100 insert in order to read out from each end of the sequence into the remainder of the gene. Sequencing by the dideoxy method of Sanger *et al.* (1977) was performed on the lambda templates directly, with the use of a fmol DNA sequencing kit from Promega. As new sequence was

determined, new primers were synthesized and used to 'walk' along the clones to obtain a complete open reading frame (ORF). Sequencing on the opposite strand using another set of primers confirmed the sequence. A complete sequence was considered to be obtained following translation of the ORFs and comparison to *S. cerevisiae* and *Schizosaccharomyces pombe* topo I gene sequences. TRANSLATE, BESTFIT, PILEUP and BLAST analyses (GCG, Wisconsin Software package, UW Madison, WI) were used in this determination. The primers used for the sequence determination are given in Table 3, and are highlighted in bold type in FIG. 6.

TABLE 3

Primer No.	Nucleotide Position in FIG. 6	Sequence	SEQ ID NO:
1	56-76	5'-GCTCCGCACATTCTATTACACC-3'	12
2	353-376	5'-CCATCAATTTACTCATCAGTTTGG-3'	13
3	624-646	5'-GAAGAAGATGAAGACGAAGTCCC-3'	14
4	898-918	5'-GGTGGGAAGTGAATCAAGAAG-3'	15
5	857-879	5'-GGTTAAATCTGAAACTCCTGAAG-3'	16
6	1134-1155	5'-GATTTTTTGGAAGTTTTAAAAG-3'	17
7	1200-1221	5'-GATTTTAGTAAAATGTATGCTC-3'	18
8	1410-1428	5'-GGGAAATTGAAACGTCGAG-3'	19
9	2074-2096	5'-CCATGCAAGATCAAATTGATATA-3'	20
10	2286-2307	5'-GAACCCAAATTGAAAAAGAAAG-3'	21
11	2442-2466	5'-GGTAAACCATTATTAACTGAATCAG-3'	22
12	2617-2640	5'-CAATTCAATTAAAAGATAAAGAAG-3'	23
13	2870-2891	5'-GTACCTGTAATATATATCCAC-3'	24*
14	2914-2937	5'-CCTTTCACACTGCAGTATCAATC-3'	25*
15	2800-2819	5'-CCCCTAATTTTAGAATCTCC-3'	26
16	2640-2662	5'-CCTAAAGAAACTTCAGAATTATC-3'	27
17	2397-2418	5'-CACGTTCTAATTTTTTTTTAGC-3'	28
18	2142-2162	5'-AGCCACCGTTCTATTGGCAGC-3'	29
19	1893-1911	5'-CAACTTCAACTTCTTGATA-3'	30
20	1142-1165	5'-CCACCACATTCTTTTAAAACTTCC-3'	31
21	978-997	5'-GGTAATGGTTCATATGGTGG-3'	32
22	924-945	5'-GCCATTTGATATAACCATCACC-3'	33
23	681-702	5'-CTTTAGTTTCGGTTTTAACTTG-3'	34
24	353-376	5'-CCAAACTGATGAGTAAATTGATGG-3'	35

* SEQ ID NO:24 and 25 have a one base deletion with respect to the actual sequence shown in FIG. 6. The primers were initally designed from partial sequence infomation and the presence of two "T"s in the actual sequence (positions 2875 and 2932 in FIG. 6) were unknown when the primers were constructed.

3b. Sequence of *C. albicans* topo I gene

The sequence of the complete open reading frame and surrounding genomic sequences determined is given in SEQ ID NO:1 shown in FIG. 6. Primers used for DNA sequencing are denoted in FIG 6. in bold letters. Potential TATA and CAAT sequences which may be involved in transcriptional initiation are italicized.

3c. Comparative homology of *C. albicans* topo I to other eukaryotic type I topoisomerases

The PILEUP routine of the GCG software package was used to compare the predicted amino acid sequence encoded by the *C. albicans* TOP1 gene with that of topoisomerase I from *S. cerevisiae* (Thrash *et al.*, 1985), *S. pombe* (Uemura *et al.*, 1987) and human cells (D'Arpa *et al.*, 1988). The predicted amino acid sequence determined for *C. albicans* topo I shows 62% overall identity to the predicted amino acid sequence of *S. cerevisiae* topo I and 45% overall identity to the predicted amino acid sequence of the human topo I.

3d. Amino acid sequences specific to *C. albicans* topo I.

Several regions of amino acids in the topo I sequence of *C. albicans* differ from analogous regions in human topo I. A comparision of the predicted *C. albicans* topo I sequence to the human topo I sequence was performed using the GCG PILEUP routine and is provided in FIG. 7. (According to this program, the symbol "|" indicates identical amino acids between two sequences (i.e. having a match value of 1.0) and the symbols ":" and "." represents similar amino acids between the two sequences (having match values of 0.5 and 0.1 respectively). As seen in FIG. 7, the 5' portion of *C. albicans* TOP1 gene encodes a stretch of charged, hydrophilic amino acids (i.e. from about amino acid position 1 to about position 150) which differ from the amino acids in the 5' amino terminal domain of human topo I. Thus the 5' amino terminal domain of the *C. albicans* enzyme (or nucleotide sequence) may provide a peptide (or nucleic acid) marker specific for *Candida* species.

The predicted amino acid sequence of the *C. albicans* topoisomerase I also differs

from the human amino acid sequence in two regions which are associated with camptothecin-resistance in mutants of topoisomerase I from human or *S. cerevisiae* (Fujimori *et al.*, 1994; Levin *et al.*, 1993), and may contribute to the difference in camptothecin sensitivity seen between topoisomerase I from human and *C. albicans* (Fostel et al, 1992). Camptothecin has been shown to stabilize the topoisomerase I/DNA cleavage complex, and this stabilization is thought to be the mode of action of this cytotoxic and fungicidal compound. Regions of the topoisomerase protein which are correlated with resistance to camptothecin may be important targets for fungal specific topoisomerase stabilizing agents.

Two positions associated with camptothecin-resistance in other topoisomerase I genes correspond to (1) the THR residue at position 309 (corresponding to one change observed in a camptothecin-resistant variant of human topoisomerase I reported by Fujimori *et al.*, 1994) and (2) the stretch of amino acids from 728 to 734 which encompasses the active site, and which differs from the homologous human topoisomerase sequence in 2 of the 7 amino acids. Changes in amino acids in this region have also been reported to correlate with camptothecin resistance in *S. cerevisiae* (Levin *et al.*, 1993)

It should be noted that other segments of the *C. albicans* topo I which do not have homologous partners in the human enzyme may also be important for therapeutic targeting of the *Candida* topoisomerase. There are four regions of this nature, specifically, the amino acids corresponding to positions 573-594, 609-633, 653-677 and 706-712 shown in FIG. 7. Not only are these fungal-specific segments, they are also situated between two segments predicted to be important for catalysis. Thus, their position in the molecule may allow these fungal-specific elements to modulate the enzyme structure or function close to the catalytic center.

Although it is possible to find amino acid correspondence between the shorter segment in the human gene and the stretch of fungal sequences between 573-712, these correspondences are not strong. Thus, the entire stretch from amino acid 573-712 is structurally different from the shorter stretch in the corresponding position of the human gene. In addition, the particular amino acids which make up this structure differ among *C. albicans*, *S. cerevisiae*, and *S. pombe*.

The active site of other type I topoisomerases contains a TYR residue which becomes covalently linked to DNA when the catalytic reaction is interrupted (Eng *et al.*, 1989, Lynn *et al.*, 1989). There is a TYR residue at the analogous position in the *C. albicans* topo I (residue 733 in FIG. 7). The short amino acid sequence surrounding this residue (i.e. SK-X-NY) is conserved in other eukaryotic type I topoisomerases; only the *C. albicans* topo I gene encodes a MET residue (i.e. SKMNY) rather than the highly conserved ILE or LEU found in other type I topoisomerases (i.e SKINY or SKLNY).

Amino acids 297-529 span a region of *C. albicans* topo I showing high homology to other eukaryotic topoisomerases. Evidence from other sources suggests that this region contains three domains which in addition to the active site (i.e. the region containing the SK-X-NY motif), are likely to be important to the catalytic function of topo I. (Gupta, M. *et al.*, 1995).

EXAMPLE 4

Expression of *C. albicans* topo I gene in *S. cerevisiae*

4a. Subcloning of *C. albicans* topo I gene in an *S. cerevisiae* expression vector

The *C. albicans* topo I gene was subcloned into an *S. cerevisiae* expression vector using standard methods of recombinant DNA technology according to the schematic outline shown in FIG. 9. The C. *albicans* topo I gene was excised from the lambda clone #26 and moved into the pVT100-U *S. cerevisiae* expression vector (Thierry *et al.*, 1987), which allows the *C. albicans* gene to be under the control of the *S. cerevisiae* alcohol dehydrogenase 1 (ADH1) promoter. The majority of the *C. albicans* topoisomerase I gene was excised by cutting the lambda clone #26 with Xba I and Pvu II. This DNA fragment is missing short fragments of the 5' and 3' ends of the open reading frame, so an adapter DNA containing the missing sequences from the 5' and 3' ends of the gene was synthesized. The sequence of the adapter is given in FIG. 8.

The first step in the construction was to insert the adapter DNA into pVT100-U. For this construction, pVT100-U was digested with HinDIII and BamH1 and electrophoresed on an 0.8% low melting point agarose gel (LMPA, GIBCO BRL, Gaithersburg, MD). The agarose slice containing the DNA was melted, added to ligation mix containing the hybridized adapter oligonucleotides (see FIG. 8) with T4 DNA ligase and incubated at 16°C for 24 hours. Competent DH5α *E. coli* (GIBCO BRL, Gaithersburg, MD) were then transformed with the reaction mix. Plasmid DNA was isolated as described by Sambrook *et al.* (1989), and restricted with EcoRI to identify clones containing the adapter DNA. Clone pVT100-UCT7 contained the adapter DNA by this criterion.

The next step in the construction was to insert the *C. albicans* gene in the adapter in pVT100-UCT7. To do this, pVT100-UCT7 was cut with Xba I and Pvu II and electrophoresed on an 0.8% LMPA gel. Lambda clone #26 carrying the *C. albicans* insert was prepared as described by Sambrook *et al.* (1989), and also restricted with Xba I and Pvu II. The lambda DNA was electrophoresed on LMPA, and the agarose containing a 2.3 kb band excised and ligated with T4 DNA ligase at 16° C for 24 hours to the cut pVT100-UCT7 DNA. The ligation mixture was used to transform competent DH5α *E. coli* (GIBCO

BRL, Gaithersburg, MD). Plasmid DNA was prepared from several clones as before and digested with Xba I and Pvu II to verify the presence of the 2.3 kb fragment of *C. albicans* DNA. Clone pVT100-UCT7-7, containing this fragment, was selected for further analysis. A culture of *E. coli* DH5α which contains pVT100-UCT7-7 was deposited under the terms of the Budapest Treaty with the Agricultural Research Culture Collection, Peoria, Illinois, on October 11, 1995, and has received the accession number NRRL-B-21504.

4b. Expression of the gene encoding *C. albicans* topo I in *S. cerevisiae*

DNA from clone pVT100-UCT7-7 was prepared from a 100 mL culture of *E. coli* using a Wizard Maxi-prep kit obtained from Promega (Madison, WI) and used to transform *S. cerevisiae* strains JN362a t1 and JN394 t1 using the method of Schiestl and Gietz (1989). Strains were obtained from Dr. John Nitiss, Children's Hospital, Los Angeles; genotypes are as given below:

JN362a t1: MATa ura3-52, leu2, trp1, his7, ade1, top1::LEU2 ISE2 and

JN394 t1: MATa ura3-52, leu2, trp1, his7, ade1, top1::LEU2 rad52::TRP1 ISE2.

The ISE mutation alters cell permeability thereby allowing the strain to be measured for sensitivity to topoisomerase agents such as camptothecin (Nitiss and Wang, 1988). The topI::LEU 2 mutation results in the elimination of functional topo I activity.

Approximately 5 µg of pVT100-UCT7-7 DNA was used for each transformation with 50 µg sonicated denatured salmon sperm DNA (obtained from Dr. Robert Simmer, Abbott Laboratories) added as carrier. Other reagents were obtained from Bio101 (Vista, CA). Cells were selected on SD-ura media (0.67 % w/v Yeast Nitrogen Base from Difco, Detroit, MI; 0.2 % w/v CSM - ura from Bio101, Vista, CA; 2% glucose from Difco, Detroit, MI; 25 mM Hepes, pH 7.2, from Sigma, St. Louis, MO). Ten transformants were selected for further testing, and where determined, showed similar characteristics. Cells carrying pVT100-UCT7-7 grew with similar rates as parent cells carrying pVT100-U (no topo I).

The sensitivity of the transformed cells to camptothecin was determined as one measure of topoisomerase activity. These cells have been shown to be sensitive to camptothecin only when they contain a functional topoisomerase (Nitiss and Wang, 1988). Cells were grown overnight in SD-ura, diluted to a density of 5×10^4 cells/mL as determined by microscopic examination using a hemocytometer, and 200 µL plated in each well of a microtiter plate (Becton Dickinson and Co., Lincoln Park, NJ). Test wells also received 4 µL of different solutions of camptothecin (Sigma, St. Louis, MO) dissolved in DMSO. The minimal inhibitory concentrations for 50% growth inhibition (MIC_{50}) was determined by visual observation after three days of growth at 30°C, and are given in Table 4.

As shown in Table 4, strains JN362 tl and JN 394 tl, either alone or carrying only the pVT100-U plasmid (i.e. the expression vector lacking a TOP1 gene), were essentially resistant to camptothecin as evidenced by MIC_{50} values of greater than 100 µg/mL. In contrast, strain JN394 tl carrying either the *C. albicans* TOP1 gene (in pVT100-UCT7-7), the *S. cerevisiae* TOP1 gene under the control of the ADH1 promoter (in pVT100-UBHT-5) or the human TOP1 gene (in pVT100::HsTOP1) showed MIC_{50} values of 0.3, 0.3 and 0.005 µg/mL respectively indicating that these strains were sensitive to camptothecin. Similarly, strain JN362 tl carrying either pVT100-UBHT-5 or PVT100::HsTOP1 showed sensitivity to camptothecin as evidenced by MIC_{50} values of 10.0 and 0.2 µg/mL respectively. A transient inhibition of growth was also observed in strain JN 362a tl carrying pVT100-UCT7-7 indicating a partial or lesser degree of sensitivity to camptothecin. For comparison, strain JN394 (having a wild-type level of topo I) was shown to have an MIC_{50} of 1.56 µg/mL.

TABLE 4

MIC_{50} values (in µg/mL) for *S. cerevisiae* strains expressing different TOP1 genes.

	No Plasmid	pVT100-U	pVT100-UCT7-7	pVT100-UBHT-5	pVT100::HsTOP1
JN 362a tl	>100.0	>100.0	>100.0*	10.0	0.2
JN 394 tl	>100.0	>100.0	0.3	0.3	0.005
JN 394	1.56	-	-	-	-

*transient inhibition of growth was observed.

4c. Characterization of recombinant *C. albicans* topo I activity

Cells from frozen stocks were streaked on SD-ura with 2% Bacto agar (Difco, Detroit, MI). Individual colonies were used to inoculate 100 mL of SD-ura, grown overnight, then used to inoculate 750 mL cultures at approximately 10^5 cells per mL. After growth for 16 hours, cells were harvested by centrifugation, washed with TGE buffer, and frozen on dry ice. Cells were thawed and lysed by vortexing in the presence of an equal volume of washed .5 mm diameter glass beads (Biospec Products, Inc., Bartlesville, OK) in the presence of TGE buffer containing 1 mM PMSF (phenylmethyl-sulfonyl fluoride), 1 µg/mL aprotinin and leupeptin and 0.1 mM DTT (dithiothreitol), 0.05% v/v Tween 80, all obtained from Sigma, St. Louis, MO.

After approximately 40-45% of the cells were judged to be lysed by microscopic examination, the lysate was recovered and centrifuged at 12,000 rpm in a Sorvall SS34 rotor for 40 minutes at 6°C. The supernatant was recovered, and incubated for 140 minutes at 4°C

with hydroxylapatite resin. The resin was recovered by centrifugation in an IEC refrigerated centrifuge at 4000 rpm using a type 224 rotor for 6 minutes. The resin was washed twice with buffer G plus 0.3 M KPO_4, pH 7.5 (buffer G is 0.1 mM EDTA, 10% glycerol) and collected as before. The topoisomerase activity was eluted from the resin by incubating at room temperature for 10 minutes in the presence of buffer G plus 0.7 M KPO_4 and removing the resin by centrifugation. The eluate was dialyzed overnight at 4°C vs. 50% glycerol, 100 mM KCl, 50 mM Tris pH 7.5, 0.1 mM EDTA, 0.1 mM DTT, 1 mM PMSF and stored at -20°C.

The protein content of each lysate was determined using the method of Bradford (1976) using reagents obtained from Bio-Rad (Hercules, CA), and is given in Table 5. Topoisomerase activity was determined using standard conditions (Fostel *et al.*, 1992). Briefly, dilutions of each lysate were made in a dilution buffer containing bovine serum albumin (BSA; molecular biology grade, obtained from GIBCO BRL, Gaithersberg, MD) in buffer G with 15 mM KPO_4 pH 7.5. One µL of each dilution was added to a 20 µL reaction containing 25 mM Tris pH 7.5; 50 mM KCl; 2.5 mM $MgCl_2$; 0.5 mM EDTA; BSA at 50 mg/mL; substrate DNA (supercoiled plasmid pBR322 obtained from GIBCO BRL, Gaithersberg, MD) at 6 µg/mL. Reactions were incubated at 30°C for 15 minutes, then stopped by the addition of 3 µL loading buffer (0.67% SDS; 67 mM EDTA; 26.7% sucrose; 0.067% each bromphenol blue and xylene cyanol FF obtained from Sigma, St. Louis, MO), and analyzed by visual inspection after electrophoresis on a 1% agarose gel in TBE buffer (from Mallinckrodt GenAR, Paris, KY). One unit of activity (U)was defined as that needed to relax 50% of the substrate DNA under these conditions. Activity measures are also given in Table 5 below.

TABLE 5

Strain	Plasmid	Volume (µL)	Protein (µg/µL)	Topoisomerase (U/µL)	Specific Activity (U/µg)
JN 362a tl	pVT100-U	315	6.1	<1	<0.16
JN 362a tl	pVT100-UCT7-7	205	10.7	1000	93
JN 362a tl	pVT100-UBHT-5	225	6.5	300	46

As Table 5 indicates, the presence of the *C. albicans* gene on pVT100-UCT7-7 correlates with topoisomerase activity in the cell.

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We claim:

1. An isolated and purified single- or double-stranded polynucleotide selected from the group consisting of

a) a polynucleotide having a nucleotide sequence comprising the sense sequence of SEQ ID NO:1 from about nucleotide position 345 to about nucleotide position 2807;

b) a polynucleotide having a nucleotide sequence comprising the sense sequence of SEQ ID NO:1 from about nucleotide position 474 to about nucleotide position 2807;

c) a polynucleotide having a nucleotide sequence comprising the sense sequence of SEQ ID NO:1 from about nucleotide position 474 to about nucleotide position 923;

d) a polynucleotide having a nucleotide sequence comprising the sense sequence of SEQ ID NO:1 from about nucleotide position 2199 to about nucleotide position 2624;

e) a polynucleotide having a nucleotide sequence comprising the sense sequence of SEQ ID NO:1 from about nucleotide position 2655 to about nucleotide position 2807;

f) a polynucleotide complementary to one of the polynucleotides of (a), (b), (c), (d) and (e);

g) a polynucleotide that, on expression, encodes a polypeptide encoded by one of the polynucleotide of (a), (b), (c), (d) and (e); and

h) a polynucleotide that hybridizes, under stringent conditions, to at least one of the polynucleotides of (a), (b), (c), (d), (e), (f) and (g).

2. An expression vector comprising an isolated and purified polynucleotide of Claim 1 wherein the polynucleotide is a DNA molecule.

3. A host cell transformed with the expression vector of Claim 2.

4. The transformed host cell of Claim 3 that is a eukaryotic cell.

5. A recombinant *C. albicans* topoisomerase I.

6. The topoisomerase of Claim 5 having the amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

7. An isolated and purified polypeptide of fewer than about 821 amino acid residues having an amino acid sequence comprising at least one of

a) the sequence from residue position 1 to residue position 150 of SEQ ID NO:3;

b) the sequence from residue position 573 to residue position 717 of SEQ ID NO:3; and

c) the sequence from residue position 728 to residue position 778 of SEQ ID NO:3.

8. A method for identifying antifungal activity in a test compound comprising the steps of:

(a) providing a first living cell and a second living cell;

(b) altering said first living cell to produce a modified living cell, wherein said modified living cell has an increased level of topoisomerase I activity relative to said second living cell;

(c) exposing said modified living cell and said second living cell to said test compound under conditions suitable for cell growth; and

(d) comparing the extent of cell mortality or attentuated cell growth of said modified living cell to said second living cell.

9. The method of Claim 8 wherein said step of altering said first living cell is achieved by introducing into said first living cell an expression vector or plasmid comprising a polynucleotide having a nucleotide sequence comprising the sequence of SEQ ID NO:1 from about nucleotide position 474 to about nucleotide position 2807.

10. A method for identifying antifungal activity in a compound comprising the steps of:

(a) providing a first living cell and a second living cell;

(b) altering said first living cell to produce a modified living cell, wherein said modified living cell has a decreased level of topoisomerase I activity relative to said second living cell;

(c) exposing said modified living cell and said second living cell to said test compound under conditions suitable for cell growth; and

(d) comparing the extent of cell mortality or attentuated cell growth of said modified living cell to said second living cell.

11. The method of Claim 10 wherein said step of altering is achieved by disrupting a

coding region of a chromosomal topoisomerase I gene in said first living cell by introducing into said first cell a polynucleotide comprising a selectable marker gene cloned within a polynucleotide having a nucleotide sequence which comprises a sequence selected from the group consisting of

(a) SEQ ID NO:1 from nucleotide position 345 to nucleotide position 2807;

(b) SEQ ID NO:1 from nucleotide position 474 to nucleotide position 2807; and (c) a fragment of (a) or (b).

12. The method of Claims 8 or 10 wherein said first living cell and said second living cell are both derived from the same strain of *S. cerevisiae* or *C. albicans* .

FIG 1

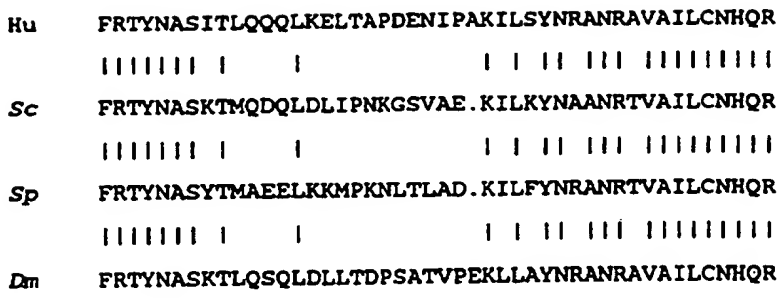
Hu FRTYNASITLQQQLKELTAPDENIPAKILSYNRANRAVAILCNHQR
Sc FRTYNASKTMQDQLDLIPNKGSVAE.KILKYNAANRTVAILCNHQR
Sp FRTYNASYTMAEELKKMPKNLTLAD.KILFYNRANRTVAILCNHQR
Dm FRTYNASKTLQSQLDLLTDPSATVPEKLLAYNRANRAVAILCNHQR

FIG. 2

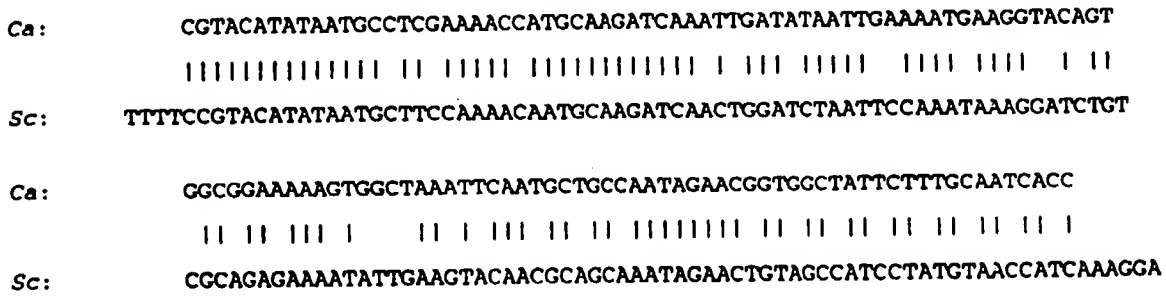

FIG. 3

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Ca  .... RTYNASKTMQ DQI....D.I IENEGTVAEK V.......AK FNAANRTVAI LCNH..
Sc   KVF RTYNASKTMQ DQL....D.L IPNKGSVAEK I.......LK YNAANRTVAI LCNHQRT
Sp   KVF RTYNASYTMA EEL....KKM .PKNLTLADK I.......LF YNRANRTVAI LCNHQRS
Um   KVF RTYNASVTFQ GLLEQTEE.W LKSRPNAAER EINQTNLRLA YNEANRQVAI LCNHQKT
Hu   KVF RTYNASITLQ QQL....KEL TAPDENIPAK I.......LS YNRANRAVAI LCNHQRA
Mm   KVF RTYNASITLQ QQL....KEL TAPDENVPAK I.......LS YNRANRAVAI LCNHQRA
Xl   KVF RTYNASITLQ QQL....DEL TNSDDNVPAK I.......LS YNRANRAVAI LCNHQRA
Dm   KVF RTYNASKTLQ SQL....DLL TDPSATVPEK L.......LA YNRANRAVAI LCNQRSV
At   KVF RTYNASITLD EML....SQE T.KDGDVTQK I.......VV YQKANKEVAI ICNHQRT
```

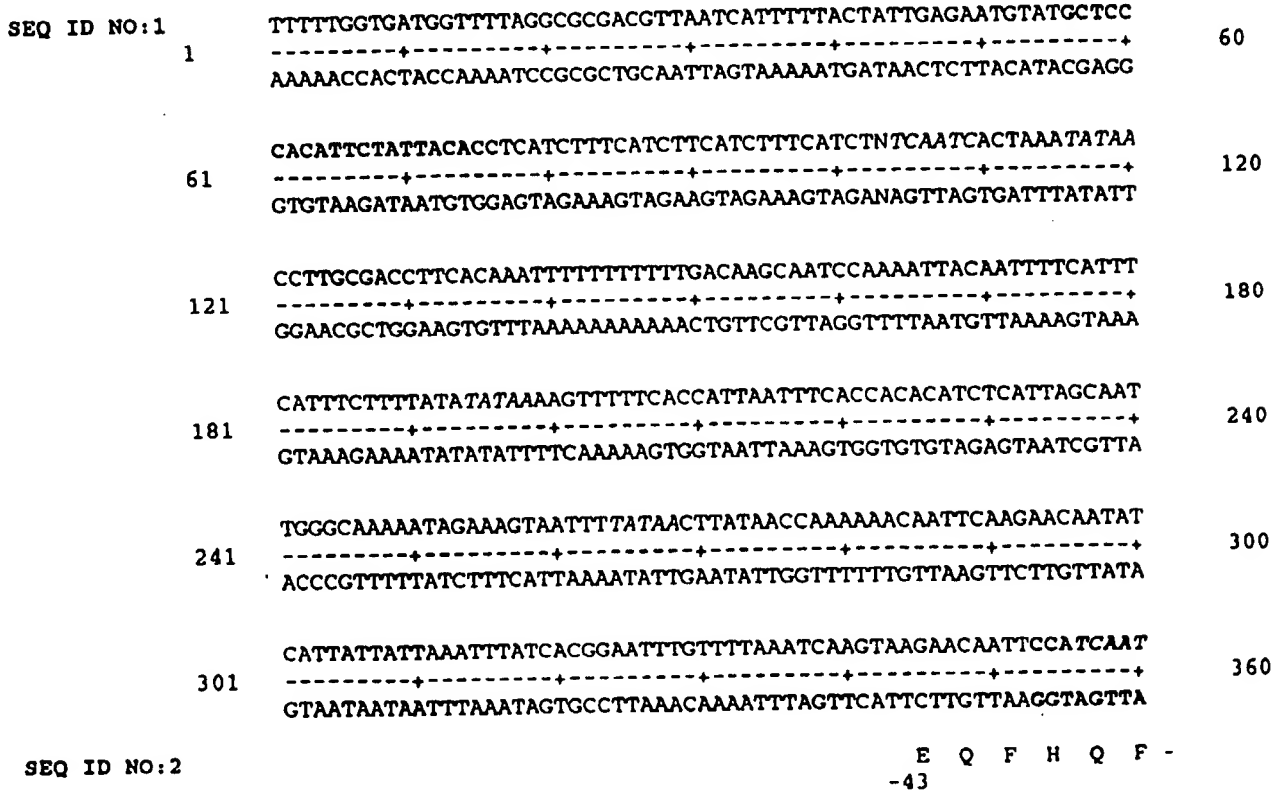
FIG. 4

Hu	IFRKNFFKDW
Mm	IFWKNFFKDW
Dm	VFNNNFFKDF
Sc	VFQKNFFNDF

FIG. 5

```
   1  AAGAATTTTT TCAATGATTT TTTGGAAGTT TTAAAAGAAT GTGGTGGTTG
  51  TGGTGTTGAA ATTAAAAAAT TTGAAAAATT AGATTTTAGT AAAATGTATG
 101  CTCATTTTGA AAAATTACGT GAAGAGAAAA AAGCCATGAG TAGAGAAGAA
 151  AAGAAAAGAA TCAAAGAAGA AAAAGAAAAA GAAGAAGAAC CTTATAGGAC
 201  TTGTTATCTT AATGGTAGAA AAGAATTAGT GGGGAATTTC CGTATTGAAC
 251  CTCCAGGTTT ATTCCGTGGT CGTGGTGCAC ATCCTAAAAC TGGGAAATTG
 301  AAACGTCGAG TAGTGCCGGA ACAAGTGACT TTGAATTTAG GTAAAGATGC
 351  TAAAATACCT GAACCACCAG CAGGTCATCA ATGGGGGGAA ATTAGACATG
 401  ATAATGAAGT CACTTGGTTG GCCATGTGGA AAGAAAATAT TTCTGATTCG
 451  TTGAAATATG TTAGATTTGC TAATAATTCA TCAGTTAAAG GACAATCTGA
 501  TTTCAAAAAA TTTGAAACGG CGAGAAAATT AAAGAGAGAT CACGTTGATT
 551  CTATTAGAAA AGATTATACC AAAATGTTAA AATCAGAGAA AATGCAAGAT
 601  AGACAAATGG CCACGGCTAT GTATCTTATT GATGTTTTTG CATTGAGGGC
 651  TGGTGGTGAA AAAGGTGAGG ATGAAGCCGA TACCGTTGGT TGTTGTTCAT
 701  TACGATATGA ACATGTAACT TTAAAACCAC CCAACAAGGT TATTTTCGAT
 751  CTTTTGGGTA AAGATTCAAT TAGATTTTAT CAAGAAGTTG AAGTTGATAA
 801  ACAAGTTTTC AAAAATCTAC GAATTTTCAA AAAATCTCCT AAACAACCTG
 851  GTGATGATTT ATTTGATCGT ATAAACCCTT CATTAGTCAA TCGACAATTA
 901  CAAAATTATA TGAAAGGATT AACAGCAAAA GTTTTCCGTA CATATAATGC
 951  CTCGAAAACC ATGCAAGATC AAATTGATAT AATTGAAAAT GAAGGTACAG
1001  TGGCGGAAAA AGTGGCTAAA TTCAATGCTG CCAATAGAAC GGTG
```

FIG. 6(a)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1
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60
61
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GTGTAAGATAATGTGGAGTAGAAAGTAGAAGTAGAAAGTAGANAGTTAGTGATTTATATT
120
121
CCTTGCGACCTTCACAAATTTTTTTTTTTGACAAGCAATCCAAAATTACAATTTTCATTT
GGAACGCTGGAAGTGTTTAAAAAAAAAAACTGTTCGTTAGGTTTTAATGTTAAAAGTAAA
180
181
CATTTCTTTTATATATAAAAGTTTTTCACCATTAATTTCACCACACATCTCATTAGCAAT
GTAAAGAAAATATATATTTTCAAAAAGTGGTAATTAAAGTGGTGTGTAGAGTAATCGTTA
240
241
TGGGCAAAAATAGAAAGTAATTTTATAACTTATAACCAAAAAACAATTCAAGAACAATAT
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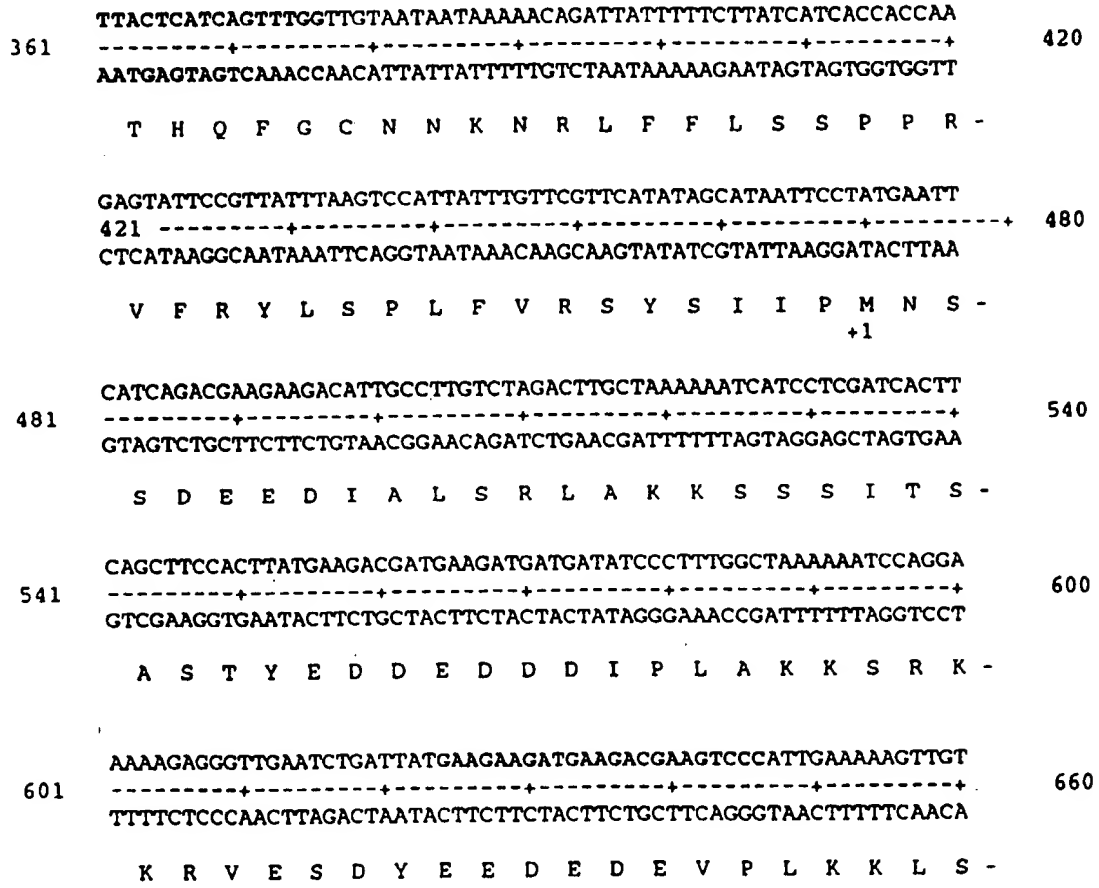
300
301
CATTATTATTAAATTTATCACGGAATTTGTTTTAAATCAAGTAAGAACAATTCCATCAAT
GTAATAATAATTTAAATAGTGCCTTAAACAAAATTTAGTTCATTCTTGTTAAGGTAGTTA
360
SEQ ID NO:2
E Q F H Q F -
-43

FIG. 6(b)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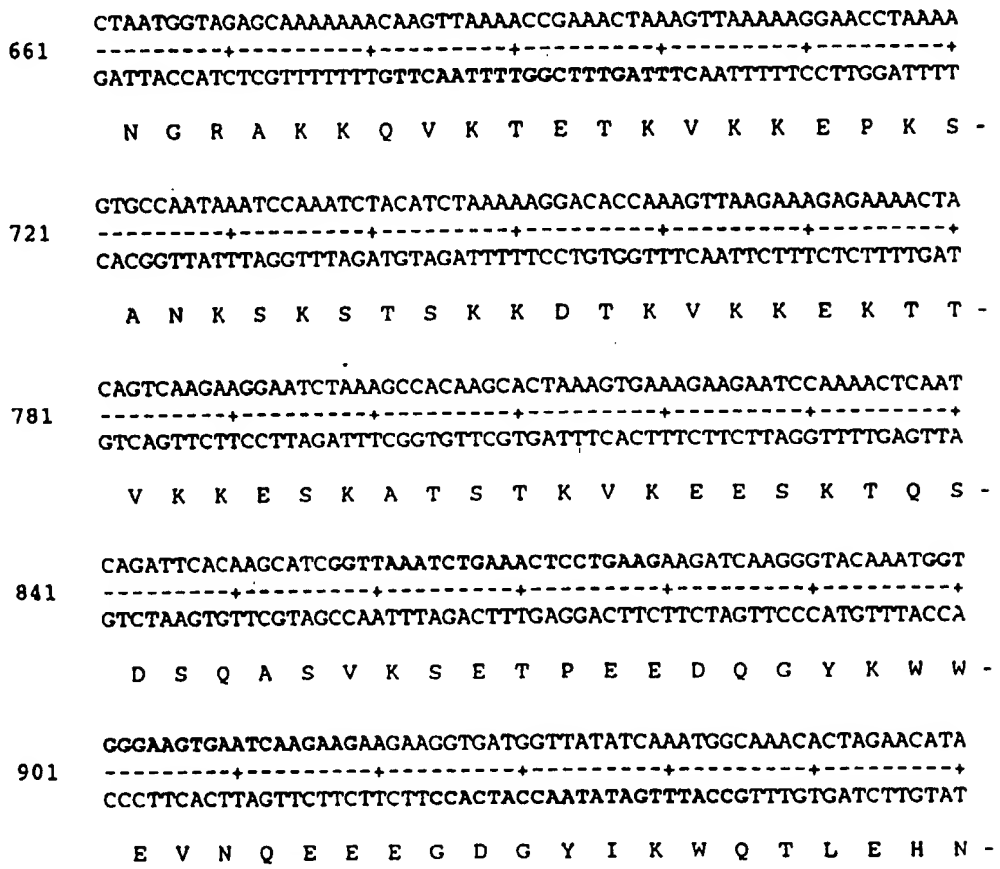
361
420
AATGAGTAGTCAAACCAACATTATTATTTTTGTCTAATAAAAAGAATAGTAGTGGTGGTT
T H Q F G C N N K N R L F F L S S P P R -
GAGTATTCCGTTATTTAAGTCCATTATTTGTTCGTTCATATAGCATAATTCCTATGAATT
421
480
CTCATAAGGCAATAAATTCAGGTAATAAACAAGCAAGTATATCGTATTAAGGATACTTAA
V F R Y L S P L F V R S Y S I I P M N S -
+1
CATCAGACGAAGAAGACATTGCCTTGTCTAGACTTGCTAAAAAATCATCCTCGATCACTT
481
540
GTAGTCTGCTTCTTCTGTAACGGAACAGATCTGAACGATTTTTTAGTAGGAGCTAGTGAA
S D E E D I A L S R L A K K S S S I T S -
CAGCTTCCACTTATGAAGACGATGAAGATGATGATATCCCTTTGGCTAAAAAATCCAGGA
541
600
GTCGAAGGTGAATACTTCTGCTACTTCTACTACTATAGGGAAACCGATTTTTTAGGTCCT
A S T Y E D D E D D D I P L A K K S R K -
AAAAGAGGGTTGAATCTGATTATGAAGAAGATGAAGACGAAGTCCCATTGAAAAAGTTGT
601
660
TTTTCTCCCAACTTAGACTAATACTTCTTCTACTTCTGCTTCAGGGTAACTTTTTCAACA
K R V E S D Y E E D E D E V P L K K L S -

FIG. 6(c)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661
720
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N G R A K K Q V K T E T K V K K E P K S -
GTGCCAATAAATCCAAATCTACATCTAAAAAGGACACCAAAGTTAAGAAAGAGAAAACTA
721
780
CACGGTTATTTAGGTTTAGATGTAGATTTTTCCTGTGGTTTCAATTCTTTCTCTTTTGAT
A N K S K S T S K K D T K V K K E K T T -
CAGTCAAGAAGGAATCTAAAGCCACAAGCACTAAAGTGAAAGAAGAATCCAAAACTCAAT
781
840
GTCAGTTCTTCCTTAGATTTCGGTGTTCGTGATTTCACTTTCTTCTTAGGTTTTGAGTTA
V K K E S K A T S T K V K E E S K T Q S -
CAGATTCACAAGCATCGGTTAAATCTGAAACTCCTGAAGAAGATCAAGGGTACAAATGGT
841
900
GTCTAAGTGTTCGTAGCCAATTTAGACTTTGAGGACTTCTTCTAGTTCCCATGTTTACCA
D S Q A S V K S E T P E E D Q G Y K W W -
GGGAAGTGAATCAAGAAGAAGAAGGTGATGGTTATATCAAATGGCAAACACTAGAACATA
901
960
CCCTTCACTTAGTTCTTCTTCTTCCACTACCAATATAGTTTACCGTTTGTGATCTTGTAT
E V N Q E E E G D G Y I K W Q T L E H N -

FIG. 6(d)

```
                ACGGTGTTATGTTTCCACCACCATATGAACCATTACCATCTCATGTCAAATTATATTATA
961             ---------+---------+---------+---------+---------+---------+    1020
                TGCCACAATACAAAGGTGGTGGTATACTTGGTAATGGTAGAGTACAGTTTAATATAATAT

                  G  V  M  F  P  P  P  Y  E  P  L  P  S  H  V  K  L  Y  Y  N -

                ACAATAAACCAGTTAATTTACCTCCAGAAGCAGAAGAAGTTGCCGGATTTTATGGAGCAA
1021            ---------+---------+---------+---------+---------+---------+    1080
                TGTTATTTGGTCAATTAAATGGAGGTCTTCGTCTTCTTCAACGGCCTAAAATACCTCGTT

                  N  K  P  V  N  L  P  P  E  A  E  E  V  A  G  F  Y  G  A  M -

                TGTTAGAAACTGATCATGCTAAAAACCCAGTTTTCCAAAAGAATTTTTTCAATGATTTTT
1081            ---------+---------+---------+---------+---------+---------+    1140
                ACAATCTTTGACTAGTACGATTTTTGGGTCAAAAGGTTTTCTTAAAAAAGTTACTAAAAA

                  L  E  T  D  H  A  K  N  P  V  F  Q  K  N  F  F  N  D  F  L -

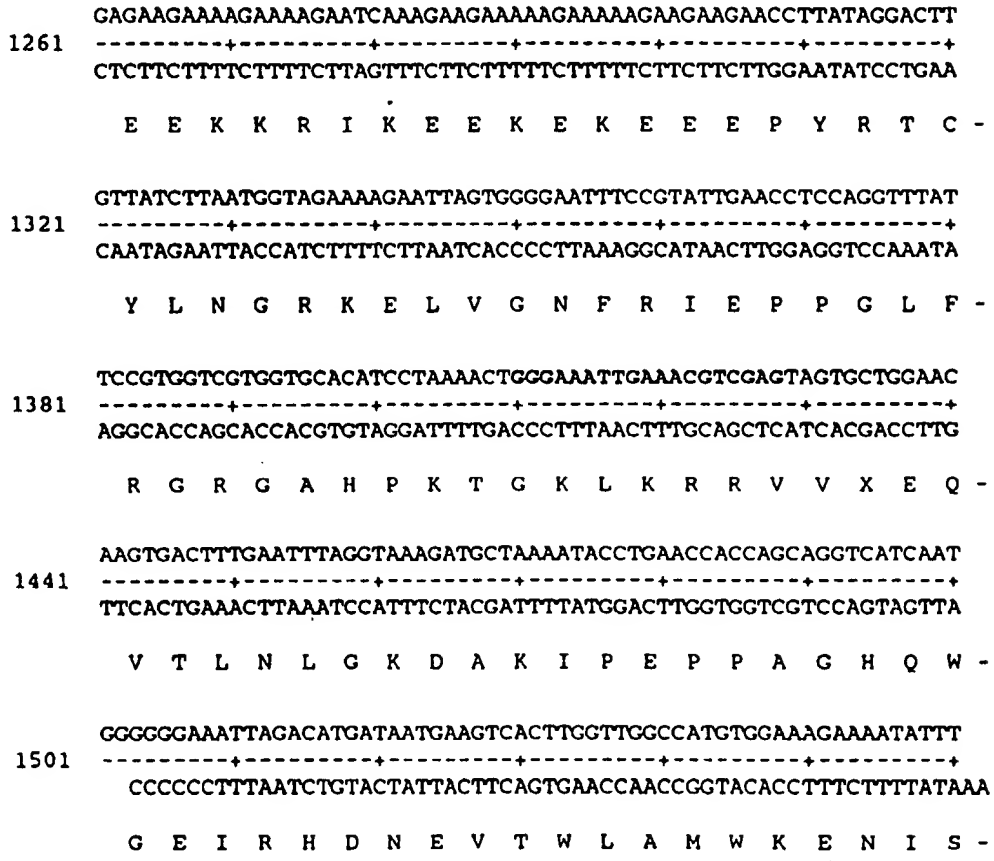
                TGGAAGTTTTAAAAGAATGTGGTGGTTGTGGTGTTGAAATTAAAAAATTTGAAAAATTAG
141             ---------+---------+---------+---------+---------+---------+    1200
                ACCTTCAAAATTTTCTTACACCACCAACACCACAACTTTAATTTTTTAAACTTTTTAATC

                  E  V  L  K  E  C  G  G  C  G  V  E  I  K  K  F  E  K  L  D -

                ATTTTAGTAAAATGTATGCTCATTTTGAAAAATTACGTGAAGAGAAAAAAGCCATGAGTA
1201            ---------+---------+---------+---------+---------+---------+    1260
                TAAAATCATTTTACATACGAGTAAAACTTTTTAATGCACTTCTCTTTTTTCGGTACTCAT

                  F  S  K  M  Y  A  H  F  E  K  L  R  E  E  K  K  A  M  S  R -
```

FIG. 6(e)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1261
1320
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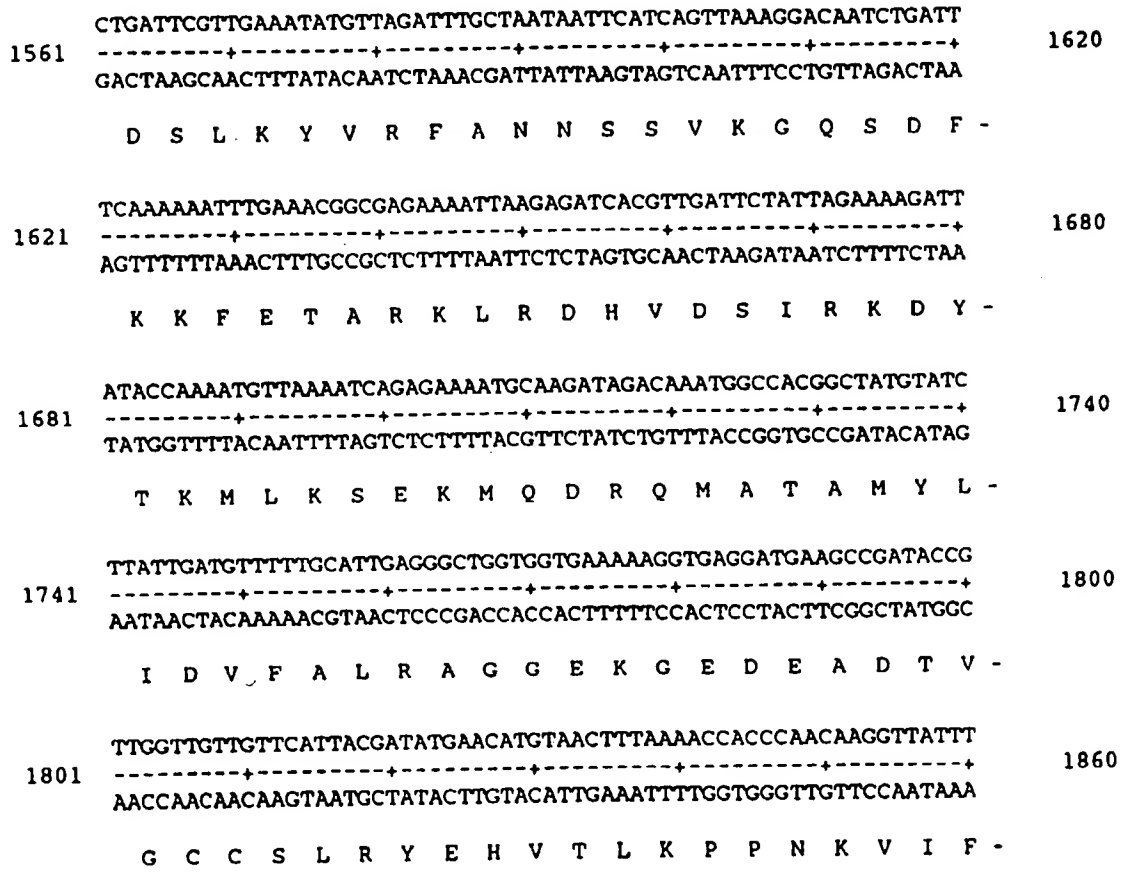
E E K K R I K E E K E K E E E P Y R T C -
GTTATCTTAATGGTAGAAAAGAATTAGTGGGGAATTTCCGTATTGAACCTCCAGGTTTAT
1321
1380
CAATAGAATTACCATCTTTTCTTAATCACCCCTTAAAGGCATAACTTGGAGGTCCAAATA
Y L N G R K E L V G N F R I E P P G L F -
TCCGTGGTCGTGGTGCACATCCTAAAACTGGGAAATTGAAACGTCGAGTAGTGCTGGAAC
1381
1440
AGGCACCAGCACCACGTGTAGGATTTTGACCCTTTAACTTTGCAGCTCATCACGACCTTG
R G R G A H P K T G K L K R R V V X E Q -
AAGTGACTTTGAATTTAGGTAAAGATGCTAAAATACCTGAACCACCAGCAGGTCATCAAT
1441
1500
TTCACTGAAACTTAAATCCATTTCTACGATTTTATGGACTTGGTGGTCGTCCAGTAGTTA
V T L N L G K D A K I P E P P A G H Q W -
GGGGGGAAATTAGACATGATAATGAAGTCACTTGGTTGGCCATGTGGAAAGAAAATATTT
1501
1560
CCCCCCTTTAATCTGTACTATTACTTCAGTGAACCAACCGGTACACCTTTCTTTTATAAA
G E I R H D N E V T W L A M W K E N I S -

FIG. 6(f)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1561
1620
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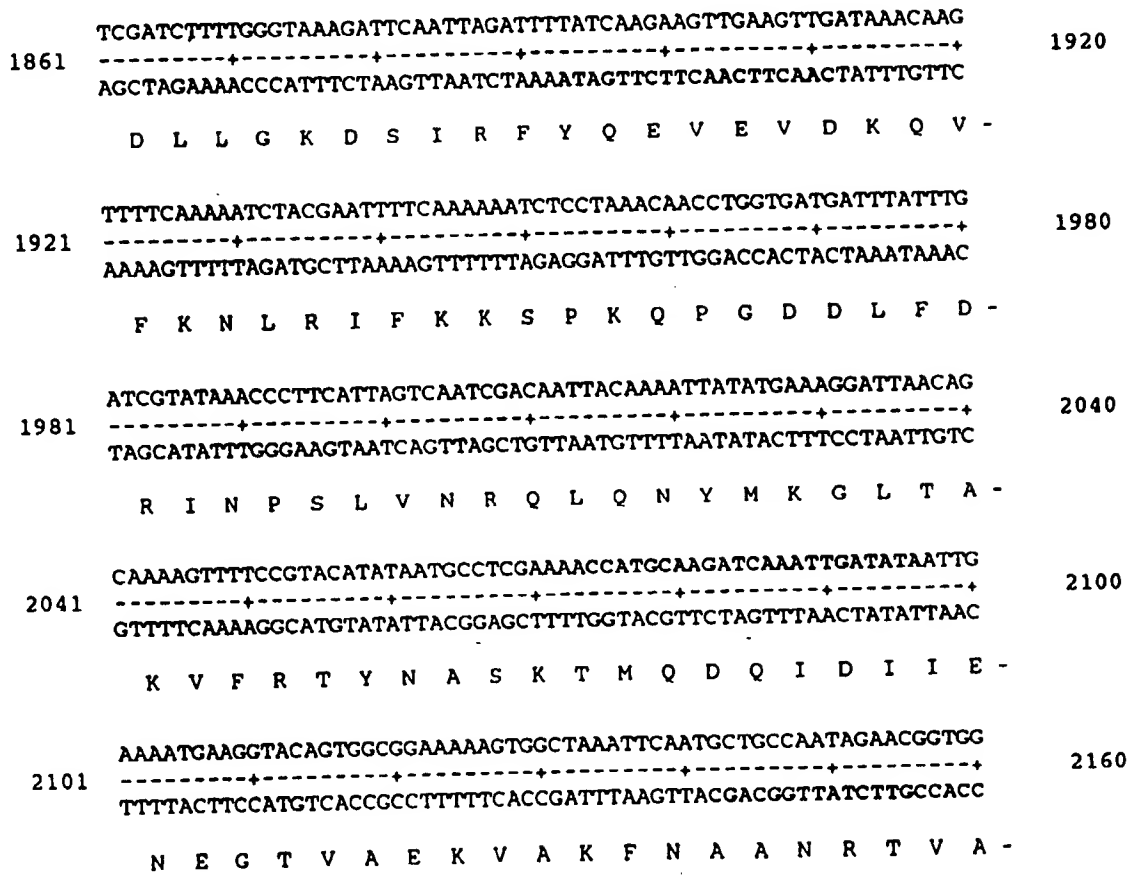
D S L K Y V R F A N N S S V K G Q S D F -
TCAAAAAATTTGAAACGGCGAGAAAATTAAGAGATCACGTTGATTCTATTAGAAAAGATT
1621
1680
AGTTTTTTAAACTTTGCCGCTCTTTTAATTCTCTAGTGCAACTAAGATAATCTTTTCTAA
K K F E T A R K L R D H V D S I R K D Y -
ATACCAAAATGTTAAAATCAGAGAAAATGCAAGATAGACAAATGGCCACGGCTATGTATC
1681
1740
TATGGTTTTACAATTTTAGTCTCTTTTACGTTCTATCTGTTTACCGGTGCCGATACATAG
T K M L K S E K M Q D R Q M A T A M Y L -
TTATTGATGTTTTTGCATTGAGGGCTGGTGGTGAAAAAGGTGAGGATGAAGCCGATACCG
1741
1800
AATAACTACAAAAACGTAACTCCCGACCACCACTTTTTCCACTCCTACTTCGGCTATGGC
I D V F A L R A G G E K G E D E A D T V -
TTGGTTGTTGTTCATTACGATATGAACATGTAACTTTAAAACCACCCAACAAGGTTATTT
1801
1860
AACCAACAACAAGTAATGCTATACTTGTACATTGAAATTTTGGTGGGTTGTTCCAATAAA
G C C S L R Y E H V T L K P P N K V I F -

FIG. 6(g)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AGCTAGAAAACCCATTTCTAAGTTAATCTAAAATAGTTCTTCAACTTCAACTATTTGTTC
1920
D L L G K D S I R F Y Q E V E V D K Q V -
1921
TTTTCAAAAATCTACGAATTTTCAAAAAATCTCCTAAACAACCTGGTGATGATTTATTTG
AAAAGTTTTTAGATGCTTAAAAGTTTTTTAGAGGATTTGTTGGACCACTACTAAATAAAC
1980
F K N L R I F K K S P K Q P G D D L F D -
1981
ATCGTATAAACCCTTCATTAGTCAATCGACAATTACAAAATTATATGAAAGGATTAACAG
TAGCATATTTGGGAAGTAATCAGTTAGCTGTTAATGTTTTAATATACTTTCCTAATTGTC
2040
R I N P S L V N R Q L Q N Y M K G L T A -
2041
CAAAAGTTTTCCGTACATATAATGCCTCGAAAACCATGCAAGATCAAATTGATATAATTG
GTTTTCAAAAGGCATGTATATTACGGAGCTTTTGGTACGTTCTAGTTTAACTATATTAAC
2100
K V F R T Y N A S K T M Q D Q I D I I E -
2101
AAAATGAAGGTACAGTGGCGGAAAAAGTGGCTAAATTCAATGCTGCCAATAGAACGGTGG
TTTTACTTCCATGTCACCGCCTTTTTTCACCGATTTAAGTTACGACGGTTATCTTGCCACC
2160
N E G T V A E K V A K F N A A N R T V A -

FIG. 6(h)

```
                CTATTTTATGTAATCACCAGCGTACGGTCAGTAAAACCCATGGTGATAGTGTTCAGAGAA
2161            ---------+---------+---------+---------+---------+---------+   2220
                GATAAAATACATTAGTGGTCGCATGCCAGTCATTTTGGGTACCACTATCACAAGTCTCTT

                  I  L  C  N  H  Q  R  T  V  S  K  T  H  G  D  S  V  Q  R  I -

                TTAATGACAAATTGAAAAAATTCATGTGGCAAAAGATTAGATTAAAGAAAATGATCTTAC
2221            ---------+---------+---------+---------+---------+---------+   2280
                AATTACTGTTTAACTTTTTTAAGTACACCGTTTTCTAATCTAATTTCTTTTACTAGAATG

                  N  D  K  L  K  K  F  M  W  Q  K  I  R  L  K  K  M  I  L  Q -

                AATTAGAACCCAAATTGAAAAAGAAAGATTCGAAATATTTTGAAGAAATTGATGATTTAA
2281            ---------+---------+---------+---------+---------+---------+   2340
                TTAATCTTGGGTTTAACTTTTTCTTTCTAAGCTTTATAAAACTTCTTTAACTACTAAATT

L  E  P  K  L  K  K  K  D  S  K  Y  F  E  E  I  D  D  L  I -

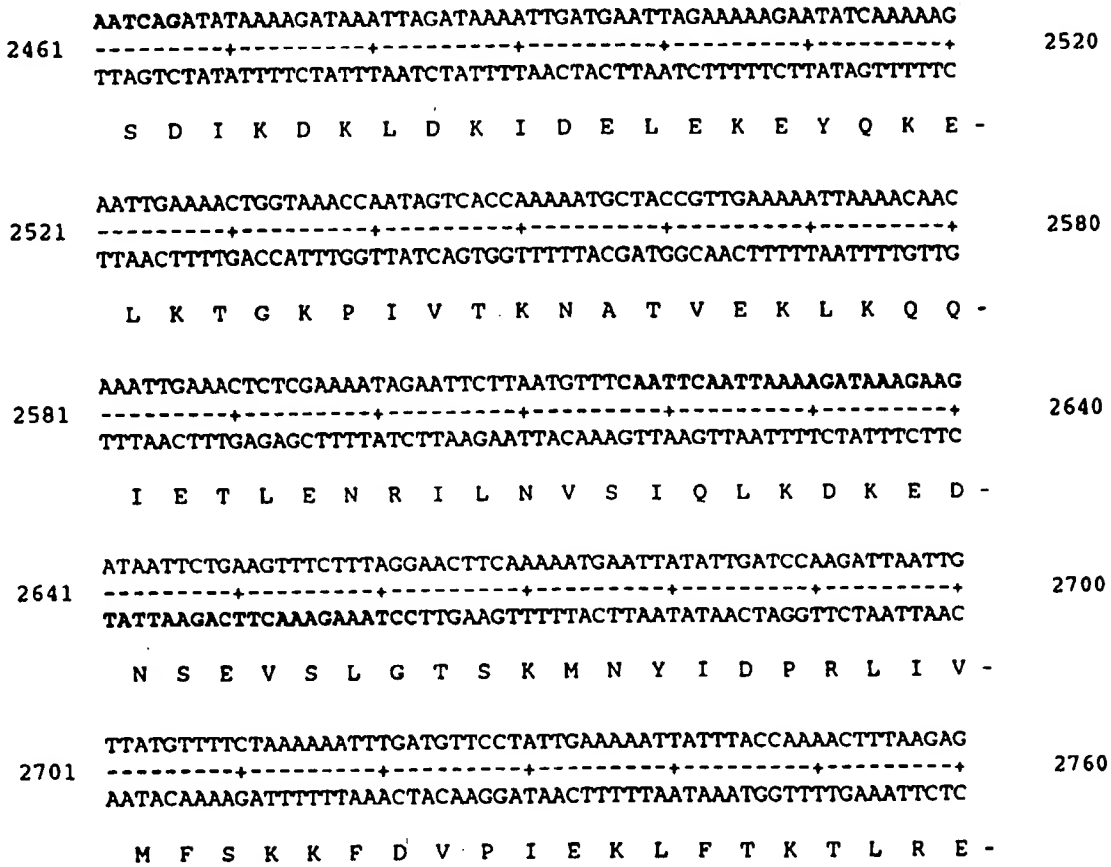
                TCAAAGAAGATATTGAACATATTCATCATACTATAATTAAAAGACAACGAGAACAAGCTA
2341            ---------+---------+---------+---------+---------+---------+   2400
                AGTTTCTTCTATAACTTGTATAAGTAGTATGATATTAATTTTCTGTTGCTCTTGTTCGAT

                  K  E  D  I  E  H  I  H  H  T  I  I  K  R  Q  R  E  Q  A  K -

                AAAAAAAATTAGAACGTGATAATGAAAAATTGAAACTTGAAGGTAAACCATTATTAACTG
2401            ---------+---------+---------+---------+---------+---------+   2460
                TTTTTTTTAATCTTGCACTATTACTTTTTAACTTTGAACTTCCATTTGGTAATAATTGAC

                  K  K  L  E  R  D  N  E  K  L  K  L  E  G  K  P  L  L  T  E -
```

FIG. 6(i)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2461
2520
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S D I K D K L D K I D E L E K E Y Q K E -
AATTGAAAACTGGTAAACCAATAGTCACCAAAAATGCTACCGTTGAAAAATTAAAACAAC
2521
2580
TTAACTTTTGACCATTTGGTTATCAGTGGTTTTTACGATGGCAACTTTTTAATTTTGTTG
L K T G K P I V T K N A T V E K L K Q Q -
AAATTGAAACTCTCGAAAATAGAATTCTTAATGTTTCAATTCAATTAAAAGATAAAGAAG
2581
2640
TTTAACTTTGAGAGCTTTTATCTTAAGAATTACAAAGTTAAGTTAATTTTCTATTTCTTC
I E T L E N R I L N V S I Q L K D K E D -
ATAATTCTGAAGTTTCTTTAGGAACTTCAAAAATGAATTATATTGATCCAAGATTAATTG
2641
2700
TATTAAGACTTCAAAGAAATCCTTGAAGTTTTTACTTAATATAACTAGGTTCTAATTAAC
N S E V S L G T S K M N Y I D P R L I V -
TTATGTTTTCTAAAAAATTTGATGTTCCTATTGAAAAATTATTTACCAAAACTTTAAGAG
2701
2760
AATACAAAAGATTTTTTAAACTACAAGGATAACTTTTTAATAAATGGTTTTGAAATTCTC
M F S K K F D V P I E K L F T K T L R E -

FIG. 6(j)

```
                AAAAGTTCATTTGGGCTATTGAATCAGCTGATGAAAATTGGAGATTCTAAAATTAGGGGT
2761            ---------+---------+---------+---------+---------+---------+  2820
                TTTTCAAGTAAACCCGATAACTTAGTCGACTACTTTTAACCTCTAAGATTTTAATCCCCA

                  K   F   I   W   A   I   E   S   A   D   E   N   W   R   F   *

                TTGTTTCTTAGCTTATTATTATATACTATATGCTGTAGAGTAAAATTTTGTACCTTGTAA
2821            ---------+---------+---------+---------+---------+---------+  2880
                AACAAAGAATCGAATAATAATATATGATATACGACATCTCATTTTAAAACATGGAACATT

                TATATATCCACTTTATTTCAACATAAAAAAATAGATTGATACTGCAGTGTGAAAAGGAAT
2881            ---------+---------+---------+---------+---------+---------+  2940
                ATATATAGGTGAAATAAAGTTGTATTTTTTTATCTAACTATGACGTCACACTTTTCCTTA

                ATGCACACACCAAGCAAGTATATTTTAGATAAAGGATTGGTGTTTTGATATTGGAAGGGC
2941            ---------+---------+---------+---------+---------+---------+  3000
                TACGTGTGTGGTTCGTTCATATAAAATCTATTTCCTAACCACAAAACTATAACCTTCCCG

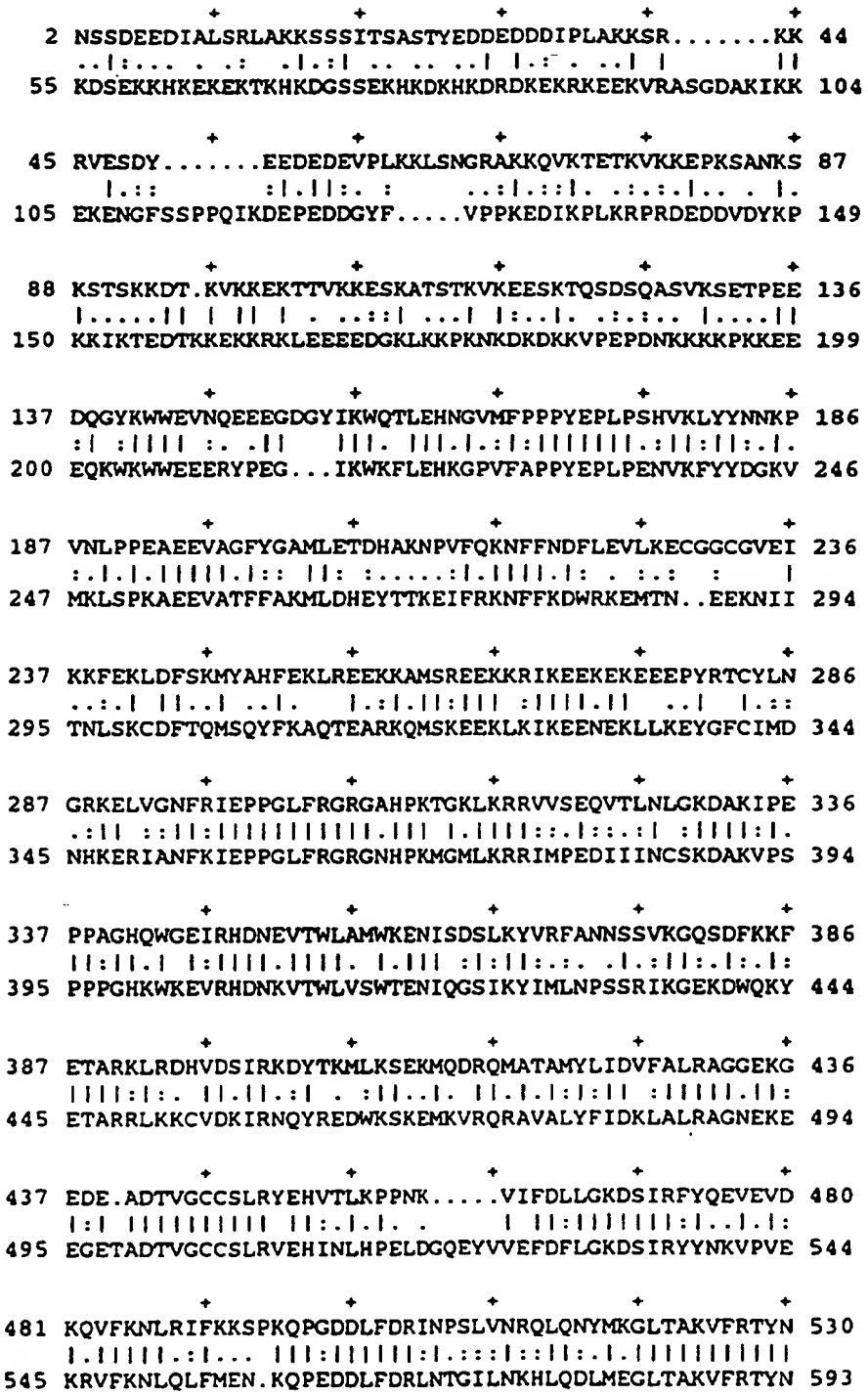
                GAAAGANGAAGGGGGGGTATCACATAGACACGTACAATCAAGAAATTGAAATTTTTCCGA
3001            ---------+---------+---------+---------+---------+---------+  3060
                CTTTCTNCTTCCCCCCCATAGTGTATCTGTGCATGTTAGTTCTTTAACTTTAAAAAGGCT

                ATTCCGTTAAATAAAAATTTTTACGCGCCTCAAATTTTCTTCTGTTTTTGTTTTTGCAAC
3061            ---------+---------+---------+---------+---------+---------+  3120
                TAAGGCAATTTATTTTTAAAAATGCGCGGAGTTTAAAAGAAGACAAAAACAAAAACGTTG

                TTCTGCAGATAGAGCAACAGTTCCCAATATCTCTATTGGAAAGATATCACAATCTCAATA
3121            ---------+---------+---------+---------+---------+---------+  3180
                AAGACGTCTATCTCGTTGTCAAGGGTTATAGAGATAACCTTTCTATAGTGTTAGAGTTAT

                GAGTAATCGTGGGAGAGTCTAATATATACGTATATACTCCTTCA
3181            ---------+---------+---------+---------+----    3224
                CTCATTAGCACCCTCTCAGATTATATATGCATATATGAGGAAGT
```

FIG. 7 (a)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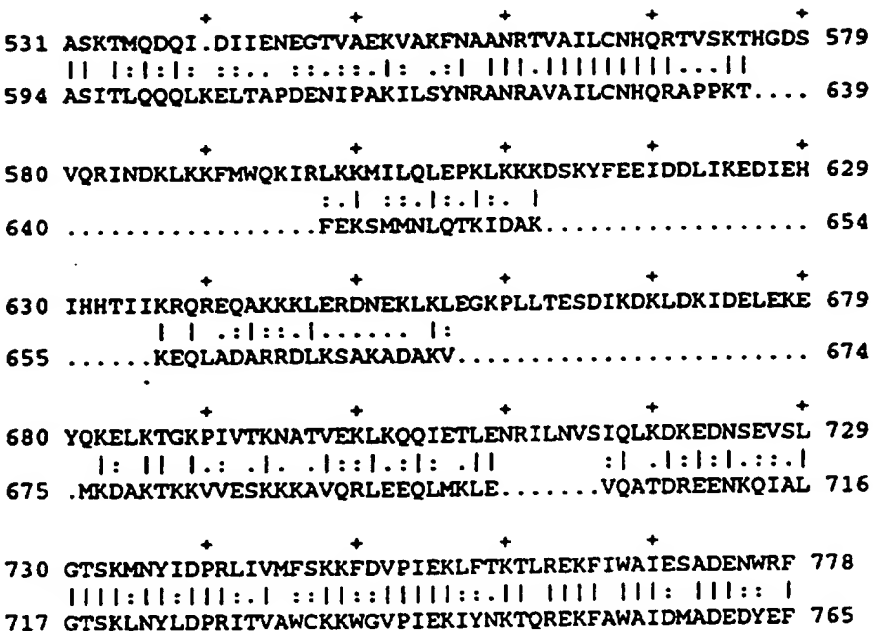
55 KDSEKKHKEKEKTKHKDGSSEKHKDKHKDRDKEKRKEEKVRASGDAKIKK 104
45 RVESDY.....EEDEDEVPLKKLSNGRAKKQVKTETKVKKEPKSANKS 87
105 EKENGFSSPPQIKDEPEDDGYF.....VPPKEDIKPLKRPRDEDDVDYKP 149
88 KSTSKKDT.KVKKEKTTVKKESKATSTKVKEESKTQSDSQASVKSETPEE 136
150 KKIKTEDTKKEKKRKLEEEEDGKLKKPKNKDKDKKVPEPDNKKKKPKKEE 199
137 DQGYKWWEVNQEEEGDGYIKWQTLEHNGVMFPPPYEPLPSHVKLYYNNKP 186
200 EQKWKWWEEERYPEG...IKWKFLEHKGPVFAPPYEPLPENVKFYYDGKV 246
187 VNLPPEAEEVAGFYGAMLETDHAKNPVFQKNFFNDFLEVLKECGGCGVEI 236
247 MKLSPKAEEVATFFAKMLDHEYTTKEIFRKNFFKDWRKEMTN..EEKNII 294
237 KKFEKLDFSKMYAHFEKLREEKKAMSREEKKRIKEEKEKEEEPYRTCYLN 286
295 TNLSKCDFTQMSQYFKAQTEARKQMSKEEKLKIKEENEKLLKEYGFCIMD 344
287 GRKELVGNFRIEPPGLFRGRGAHPKTGKLKRRVVSEQVTLNLGKDAKIPE 336
345 NHKERIANFKIEPPGLFRGRGNHPKMGMLKRRIMPEDIIINCSKDAKVPS 394
337 PPAGHQWGEIRHDNEVTWLAMWKENISDSLKYVRFANNSSVKGQSDFKKF 386
395 PPPGHKWKEVRHDNKVTWLVSWTENIQGSIKYIMLNPSSRIKGEKDWQKY 444
387 ETARKLRDHVDSIRKDYTKMLKSEKMQDRQMATAMYLIDVFALRAGGEKG 436
445 ETARRLKKCVDKIRNQYREDWKSKEMKVRQRAVALYFIDKLALRAGNEKE 494
437 EDE.ADTVGCCSLRYEHVTLKPPNK.....VIFDLLGKDSIRFYQEVEVD 480
495 EGETADTVGCCSLRVEHINLHPELDGQEYVVEFDFLGKDSIRYYNKVPVE 544
481 KQVFKNLRIFKKSPKQPGDDLFDRINPSLVNRQLQNYMKGLTAKVFRTYN 530
545 KRVFKNLQLFMEN.KQPEDDLFDRLNTGILNKHLQDLMEGLTAKVFRTYN 593

FIG.7 (b)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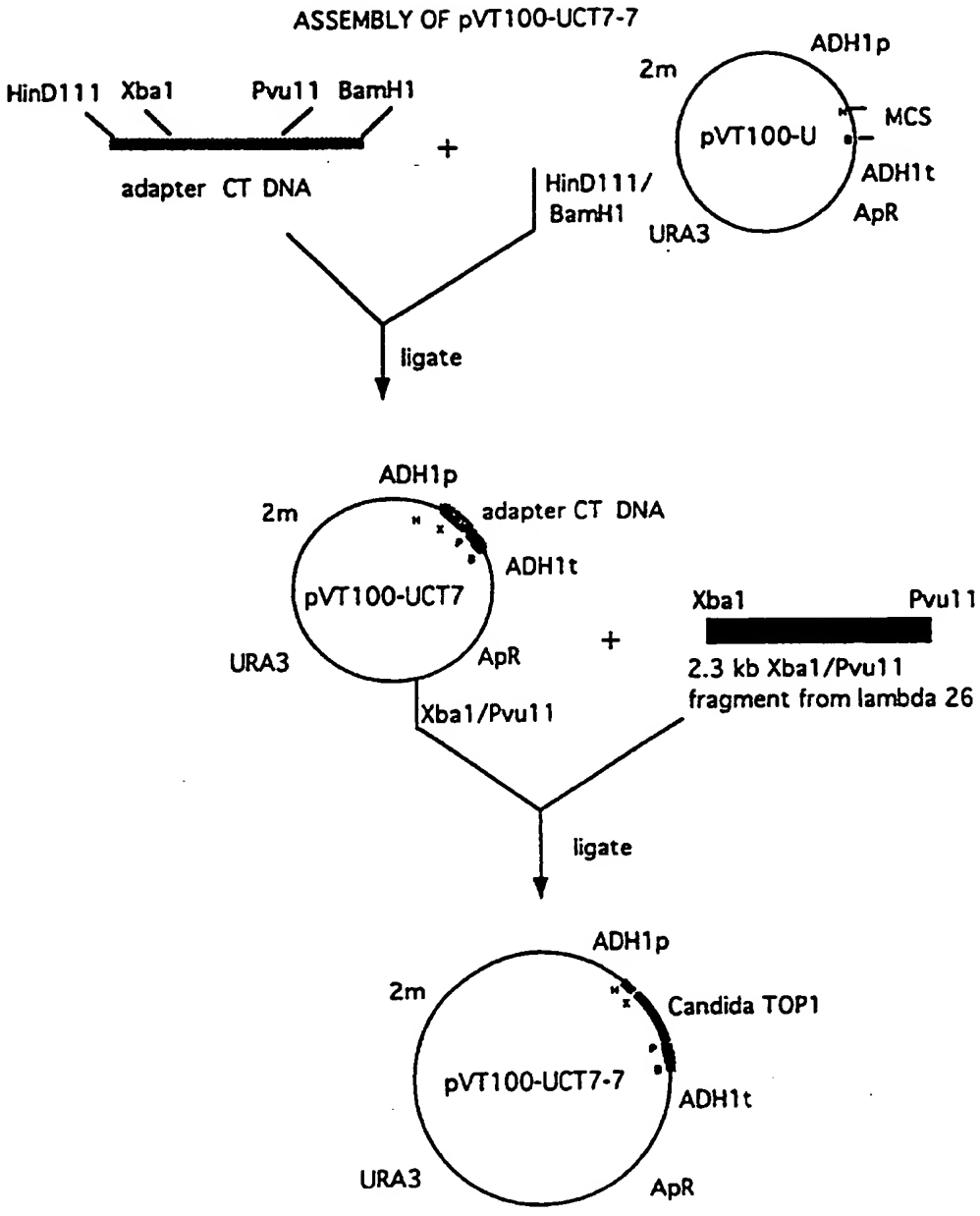
594 ASITLQQQLKELTAPDENIPAKILSYNRANRAVAILCNHQRAPPKT.... 639
580 VQRINDKLKKFMWQKIRLKKMILQLEPKLKKKDSKYFEEIDDLIKEDIEH 629
640 .....FEKSMMNLQTKIDAK..... 654
630 IHHTIIKRQREQAKKKLERDNEKLKLEGKPLLTESDIKDKLDKIDELEKE 679
655 ......KEQLADARRDLKSAKADAKV..... 674
680 YQKELKTGKPIVTKNATVEKLKQQIETLENRILNVSIQLKDKEDNSEVSL 729
675 .MKDAKTKKVVESKKKAVQRLEEQLMKLE.....VQATDREENKQIAL 716
730 GTSKMNYIDPRLIVMFSKKFDVPIEKLFTKTLREKFIWAIESADENWRF 778
717 GTSKLNYLDPRITVAWCKKWGVPIEKIYNKTQREKFAWAIDMADEDYEF 765

FIG. 8

5' AGCTTATGAATTCATCAGACGAAGAAGACATTGCCTTGTCTAGAATTCAGCTGATGAACATTGGAGATTCTAAAATTAG

3' ATCTTAAGTAGTCTGCTTCTTCTGTAACGGAACAGATCTTAAGTCGACTACTTGTAACCTCTAAGATTTTAATCCTAG

FIG. 9


ASSEMBLY OF pVT100-UCT7-7
HinD111
Xba1
Pvu11
BamH1
adapter CT DNA
+
HinD111/
BamH1
2m
ADH1p
pVT100-U
MCS
ADH1t
ApR
URA3
ligate
ADH1p
2m
adapter CT DNA
ADH1t
pVT100-UCT7
URA3
ApR
Xba1/Pvu11
+
Xba1
Pvu11
2.3 kb Xba1/Pvu11
fragment from lambda 26
ligate
ADH1p
2m
Candida TOP1
pVT100-UCT7-7
ADH1t
URA3
ApR

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(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

(72) Inventors: FOSTEL, Jennifer, M.; 7413 10th Avenue, Kenosha, WI 53143 (US). GILES, Kellie, M.; 9526 Fairway Circle, Franklin, WI 53132 (US). TAYLOR, Alison; 2032 Flower Circle, Arlington Heights, IL 60004 (US). MCGONIGAL, Thomas, P.; 3211 Rugby Court, Waukegan, IL 60087 (US). SARTHY, Aparna, V.; 4836 Dorothy Court, Waukegan, IL 60089 (US).

(74) Agents: DANCKERS, Andreas, M. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

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(57) Abstract

The present invention provides an isolated and purified polynucleotide that encodes *Candida albicans* type I topoisomerase. Methods of making recombinant *C. albicans* topoisomerase I using those polynucleotides and host cells transformed with those polynucleotides are also provided. The present invention also provides a method for identifying compounds which inhibit the growth of fungal cells using the polynucleotide.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/61 C12N15/81 C12N9/90 C12N1/19 C12Q1/02
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 117, no. 21, 23 November 1992 Columbus, Ohio, US; abstract no. 207692, FOSTEL, JENNIFER M. ET AL: "Characterization of DNA topoisomerase I from Candida albicans as a target for drug discovery" XP002034941 see abstract & ANTIMICROB. AGENTS CHEMOTHER. (1992), 36(10), 2131-8 CODEN: AMACCQ;ISSN: 0066-4804, ---	1
Y	WO 89 09222 A (UNIV JOHNS HOPKINS) 5 October 1989 see claims --- -/--	1

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHEMICAL ABSTRACTS, vol. 125, no. 7, 12 August 1996 Columbus, Ohio, US; abstract no. 77851, TAYLOR, ALISON ET AL: "Identification of the gene encoding DNA topoisomerase I from Candida albicans" XP002034942 see abstract & FEMS MICROBIOL. LETT. (1996), 138(2-3), 113-121 CODEN: FMLED7;ISSN: 0378-1097,	1-9
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INTERNATIONAL SEARCH REPORT

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Internal Application No
PCT/US 96/17291

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WO 8909222 A	05-10-89	US 5070192 A	03-12-91
		AU 3425389 A	16-10-89
WO 9640886 A	19-12-96	NONE	